(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 May 2001 (10.05.2001)

PCT

(10) International Publication Number
WO 01/32928 A2

(51) International Patent Classification[7]: C12Q 1/68, G01N 33/50

(21) International Application Number: PCT/US00/30474

(22) International Filing Date:
3 November 2000 (03.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/165,398 5 November 1999 (05.11.1999) US
60/196,571 11 April 2000 (11.04.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/32928 A2

(54) Title: METHODS OF DETERMINING INDIVIDUAL HYPERSENSITIVITY TO AN AGENT

(57) Abstract: Methods of identifying hypersensitivity in a subject by obtaining a gene expression profile of multiple genes associated with hypersensitivity of the subject suspected to be hypersensitive, and identifying in the gene expression profile of the subject a pattern of gene expression of the genes associated with hypersensitivity are disclosed. The gene expression profile of the subject may be compared with the gene expression profile of a normal individual and a hypersensitive individual. The gene expression profile of the subject that is obtained may comprise a profile of levels of mRNA or cDNA. The gene expression profile may be obtained by using an array of nucleic acid probes for the plurality of genes associated with hypersensitivity. The expression of the genes predetermined to be associated with hypersensitivity is directly related to prevention or repair of toxic damage at the tissue, organ or system level. Gene databases arrays and apparatus useful for identifying hypersensitivity in a subject are also disclosed.

METHODS OF DETERMINING INDIVIDUAL HYPERSENSITIVITY TO AN AGENT

TECHNICAL FIELD

The invention generally relates to methods, compositions and devices for identifying individuals who are hypersensitive to a given agent.

BACKGROUND OF THE INVENTION

Individuals exhibit a high degree of variability in their response to chemicals, including pharmaceutical compounds. A major concern of pharmaceutical manufacturers is that a subset of the patient population may display significant toxic side effects that cannot be predicted from preclinical studies. In many cases this hypersensitivity results in extreme, and even lethal, responses. The incidence of serious and lethal adverse reactions to drugs among hospitalized patients in the United States causes at least 100,000 deaths per year. This makes adverse responses to therapeutic drugs the fifth main cause of death in the United States.

The existence of a hypersensitive sub-population is usually only discovered after a compound has been broadly prescribed and a population base of sufficient size has had exposure to the compound. These same drugs are generally safe for the majority of individuals and most respond favorably to the desired effects of the drugs. In many cases, the same drug that may elicit severe toxic responses in a subset of the population is the best drug for the particular disease being treated. For example, clozapine is a very effective drug for treating moderate to severe depression and with the majority of patients shows no toxic side effects at the recommended doses. Yet at the same dose (usually 300 mg), approximately 1% of the patient population develop agranulocytosis, a severe blood disorder.

Many compounds have either been withdrawn from the market altogether or severely restricted in use due to severe adverse responses by a subset of the patient population. In some instances, it is known that a subset of the population is hypersensitive, and physicians are advised to be alert for indications of extreme toxic response in such patients. Exemplary compounds include tienilic acid, halothane, dihydrazine, diclofenac, fialuridine, carbamazepine, Trovan™ (trovafloxacin), Seldane™ (terfenadine), hismanol, dihydrolazine, warfarin, phenytoin, omeprazole, diazepam, haloperidol, perphenazine, perhexiline, phenformin, tolbumamide, penicillin, clozapine, aminopurine, quinidine and remoxipide.

Unfortunately, in the vast majority of these cases, there is no way of identifying a hypersensitive individual before prescribing the drugs or exposing the worker to the compound. Hypersensitive individuals are discovered the hard way; they exhibit toxic side effects that most people do not. Furthermore, since the mechanisms of toxicity are specific and usually different for each drug or compound, the hypersensitive populations are also different and specific for each drug or compound.

When an approved drug is found to elicit serious toxicity in only a subset of the population the manufacturer is usually required to tightly restrict access to the drug, carefully monitor all patients who receive the drug for toxic side effects, or withdraw it from the market altogether. A high number of compounds also fail in the late stages of development because of serious toxicity in a subset of the clinical trial population. When a drug is found to cause severe toxicity in a sub-population, besides the trauma and pain for such hypersensitive individuals, there is great financial loss incurred by the manufacturer. The cost to the manufacturer of withdrawing or restricting a compound can be billions of dollars in lost market capitalization, legal liabilities and unrecoverable research and development expenses. Adverse reactions are becoming the main challenge for pharmaceutical research and development. (Drug Discovery Today) 4:393-395 (1999). In

addition, very effective drugs are often pulled from the market and thus become unavailable to those who would benefit greatly from them.

It would save lives, decrease pain and suffering and save pharmaceutical manufacturers and consumers a great deal of money if there were a way to determine in advance which individuals were likely to experience severe toxic responses to a drug.

SUMMARY OF THE INVENTION

Disclosed herein are methods, gene databases, gene arrays, protein arrays, and devices that may be used to determine the hypersensitivity of individuals to a given agent, such as a drug or other chemical, in order to prevent toxic side effects.

In one embodiment, the invention relates to a method of identifying hypersensitivity in a subject by obtaining the gene expression profile of specific genes associated with hypersensitivity of the subject suspected to be hypersensitive and identifying in the gene expression profile of the subject a pattern of gene expression of the genes associated with hypersensitivity. The gene expression profile of the subject may be compared with the gene expression profile of individuals who have an acceptable response and compared with other hypersensitive individuals. The embodiment also includes, for example, identifying hypersensitivity to an agent in a subject, where the agent may be a pharmaceutical agent, industrial, household or other chemical or compound. Exemplary pharmaceutical agents are disclosed in Table 1.

The gene expression profile of the subject that is obtained may comprise a profile of levels of mRNA or cDNA. The gene expression profile may be obtained by using an array of nucleic acid probes complementary to the genes associated with hypersensitivity. The genes used may comprise at least two genes, at least 3, 4, 6, 7, 8, or 9 genes predetermined to be associated with hypersensitivity, and may also comprise at least 5, at least 10, at least

25, at least 50, at least 100, at least 250 or more genes determined to be associated with hypersensitivity.

Genes associated with hypersensitivity and used in this invention may, for example, comprise genes from a variety of different cell types, including, but not limited to, genes from multiple types of tissues, organs or systems or genes from a single type of tissue, organ or system. Exemplary organs and tissues include the liver, kidneys, heart, brain, thyroid, lung, pancreas, muscle, brain, testes, ovaries, spleen, stomach, intestines, colon, rectum, eyes, muscle, skin, and bone. Exemplary types of cells include liver cells such as, Kupfer cells, sinusoidal cells, ito cells, hepatocytes, bile duct epithelial cells, hepatic venule endothelial cells and sinusoidal epithelial cells.

A further embodiment encompasses the expression profile of the genes predetermined to be associated with hypersensitivity where expression of the genes is related to prevention or repair of toxic damage at the nucleotide, protein, macromolecule, organelle, cell, tissue, organ or system level.

In another embodiment, the gene expression profile may comprise a profile of protein expression levels, where the proteins are encoded by genes associated with hypersensitivity. The level of expression of the proteins may be directly related to the prevention or repair of toxic damage at the protein, nucleotide, macromolecule, organelle, cell, tissue, organ or system level. An additional embodiment includes protein expression profiles, where the proteins are encoded by genes associated with hypersensitivity, and the expression of the genes is, for example, associated with response to the presence of an agent, such as a toxic agent. Exemplary agents that can induce a characteristic profile of protein expression associated with hypersensitivity include those agents listed in Table 1.

The gene expression profile may be obtained from a sample from the subject, which sample may be from a cell or tissue sample and may comprise cells of different cell types. For gene expression, the sample may comprise, for example, white blood cells, skin, spinal

fluid or organ biopsy material. For protein expression analysis, the sample may comprise, for example, blood, tissue, urine, spinal fluid or serum.

In another embodiment, cells or tissues derived from an individual are used to establish primary cell cultures, for example fibroblasts, hepatocytes, and other examples known in the art. These primary cell cultures are then exposed to the agent. Co-cultures are also encompassed in the invention and are grown from two or more cell types that reflect, for example, the cell types involved in systemic toxicity. These co-cultures would then be exposed to the agent of interest.

In another embodiment, the gene expression profiles of samples from normal individuals, hypersensitive individuals or cell cultures are determined for individual agents using the methods herein described to determine drug-drug interactions. The gene expression profiles are compared to determine whether the multiple agents, for example two or more agents, elicit the same or similar gene expression profiles in the samples. The expression of the same or similar pattern(s) of toxic response genes for two or more compounds in either normal or hypersensitive individuals, is indicative that a drug-drug interaction, also described as a synergistic toxic effect, can be present if the agents are administered together, for example, during the same time period or in the same dose.

The genes used in the gene expression profile may include, but are not limited to, genes, and the proteins which they encode, which are associated with toxic outcomes affecting the pulmonary system, cardiovascular system, nervous system, digestive system, immune system, reproductive system, endocrine system, vision or skin. Exemplary types of toxicity include cardiotoxicity, blood toxicity, liver (hepatic) toxicity, kidney (renal) toxicity, neural toxicity, skin toxicity, immunotoxicity, and pulmonary toxicity. Exemplary genes associated with specific organ or system toxic outcomes are disclosed in Table 5.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with toxic outcomes such as, but not limited to, altered lipid

metabolism, altered thyroid function, organ hypertrophy, skin irritation, skin sensitization, tumor formation, dementia, inflammation, myelosuppression, peripheral neuropathy, necrosis, signal refractivity, spreading, transformation, retinopathy or optic atrophy.

The genes used in the gene expression profile may include, but are not limited to genes, and the proteins which they encode, which are associated with toxic outcomes affecting the digestive system or the organs and tissues which comprise the digestive system, for example, the liver, kidneys, colon, bladder, pancreas, stomach, intestines, rectum, or gallbladder.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with exemplary toxic outcomes such as, but not limited to, proteinuria, glomerulitis, nephritis, renal damage, renal failure, liver weight change, cholestasis, pancreatitis, liver steatosis, hyperplasia, fatty liver, jaundice, hepatitis, mutagenesis, or altered bile flow.

The genes used in the gene expression profile may include, but are not limited to genes, and the proteins which they encode, which are associated with toxic outcomes affecting the pulmonary system or the organs and tissues which comprise the pulmonary system, for example the lungs or trachea.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with toxic outcomes such as, but not limited to, lung fibrosis, pulmonary edema or lung airway reactivity.

The genes used in the gene expression profile may include, but are not limited to genes, and the proteins which they encode, which are associated with toxic outcomes affecting the cardiovascular and circulatory systems or the organs, fluids and tissues which comprise the cardiovascular and circulatory systems, for example, the heart, spleen, arteries, blood vessels, blood or blood cells, including genes associated with toxic outcomes associated with bone marrow.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with exemplary toxic outcomes such as, but not limited to, tachycardia, arrhythmia, leukemia, neutropenia, hematological alteration, hypotension, hypertension or agranulocytosis.

The genes used in the gene expression profile may include, but are not limited to genes, and the proteins which they encode, which are associated with toxic outcomes affecting the nervous system or the organs and tissues which comprise the nervous system, for example, the brain, spinal cord or nerves.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with toxic outcomes such as, but not limited to, neurodegeneration or neurotoxicity.

The genes used in the gene expression profile may include, but are not limited to genes, and the proteins which they encode, which are associated with toxic outcomes affecting the immune system or the organs and tissues which comprise the immune system, for example, the thymus, lymph nodes or lymph glands.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with toxic outcomes such as, but not limited to, a change in thymic weight or immunosuppression.

The genes used in the gene expression profile may include, but are not limited to genes, and the proteins which they encode, which are associated with toxic outcomes affecting the reproductive system or the organs and tissues which comprise the reproductive system, for example the testes, ovaries, fallopian tubes or uterus.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with toxic outcomes such as, but not limited to, teratogenesis, loss of fertility, alteration in sperm count, alteration in testes weight or alteration in testosterone levels.

The genes used in the gene expression profile include those genes, and the proteins which they encode, associated with cellular manifestations of toxicity such as, but not limited to, apoptosis, cell adhesion, autophagocytosis, cell division, chemotaxis, cell cycle arrest, circadian rhythm, cytokine release, differentiation, de-differentiation, mitochondrial damage, migration, mutation, oncosis, recombination, senescence, peroxisome proliferation, polyploidy, signal refractivity, spreading, transformation or necrosis.

The genes involved, and the proteins which they encode, may also include those associated with a specific ethnic group, sex or age group.

The genes or proteins used in the expression profile may also include the genes, and the proteins or amino acids which they encode, which are selected from the genes disclosed in (or genes comprising sequences disclosed in) Table 3, Table 4, Table 5, Table 6, Table 8, Table 10 and Table 11.

In another embodiment, the method includes obtaining a gene expression profile of genes comprising different cell types, of the subject, determining if the gene expression profile of the subject comprises a pattern of gene expression associated with hypersensitivity to an agent, and withholding that agent from those subjects who are hypersensitive or altering the therapy and closely monitoring the subjects who are hypersensitive for toxic effects.

In another embodiment, a method of identifying a plurality of genes associated with hypersensitivity to an agent is provided, comprising comparing the gene expression profile of cells treated with an agent with the gene expression profile of cells not treated with the agent and identifying genes that have altered expression due to exposure to the agent in the treated cells. The cells may comprise, for example, a number of different cell types and each cell type may comprise a gene associated with hypersensitivity to the agent. The cells may also comprise cells from of different cell types where all the cell types are derived from a single type of tissue, organ or system. The organs or tissues from which cell types

may be derived include, but are not limited to, the kidneys, liver, lungs, heart, brain, spleen, thyroid, bone, muscle, intestine, stomach, pancreas, testes, ovaries, colon or skin.

The invention also relates to a method of identifying genes having a pattern of differential gene expression indicative of hypersensitivity to an agent by comparing the gene expression profile of one or more cell types, for example, at least 2, at least 3, at least 4, at least 5, at least 10, at least 50, at least 100 or at least 250, of a subject known to be hypersensitive to the agent with the gene expression profile of the cell types in an individual known not to be hypersensitive to the agent and identifying genes from the two or more cell types which exhibit a pattern of differential gene expression associated with hypersensitivity to the agent.

In an alternative embodiment, the method of identifying genes having a pattern of differential gene expression indicative of hypersensitivity to an agent comprises comparing the gene expression profile of one or more cell types, for example, at least 2, at least 3, at least 4, at least 5, at least 10, at least 50, at least 100 or at least 250, of a subject known to be hypersensitive to the agent before treatment with the agent with the gene expression profile of the one or more cell types of the subject after treatment with the agent and identifying genes from the cell types having a pattern of differential gene expression associated with hypersensitivity to the agent.

In an alternative embodiment, the method of identifying proteins having a pattern of differential protein expression indicative of hypersensitivity to an agent comprises comparing the protein expression profile of one or more cell types of a subject known to be hypersensitive to the agent before treatment with the agent with the protein expression profile of the one or more cell types of the individual after treatment with the agent and identifying proteins from the cell types having a pattern of differential protein expression associated with hypersensitivity to the agent.

In another embodiment, there is provided an array for the identification of a gene expression profile indicative of a hypersensitivity to an agent which comprises gene probes,

for example, nucleic acid sequences which comprise a gene sequence associated with hypersensitivity to the agent, associated with the hypersensitivity to the agent. The genes are selected from the genes identified by methods disclosed herein or are selected from those genes disclosed in whole or in part in Table 3, Table 4, Table 5, and Tables 6, 8, 10 and 11. The array comprises for example, at least 5, at least 10, at least 25, at least 50, at least 100, at least 150, at least 250 different gene probes. Exemplary arrays include, for example, gene probes supported on glass slides or nylon membranes with fluorescent or radio labels, amplified fragment length polymorphism (AFLP) methods or Northern Blots.

The invention further encompasses a database of genes associated with hypersensitivity to an agent. The genes are those identified by methods disclosed herein or are selected from those genes disclosed in whole or in part in Table 3 and Table 4, Table 5 and Tables 6, 8, 10 and 11. The database of genes may comprise, for example, genes associated with altered lipid metabolism, cholestasis, immunosuppression, pancreatitis, agranulocytosis, tumor formation, teratogenesis, liver steatosis, apoptosis, cell adhesion, autophagocytosis, cell cycle arrest, circadian rhythm, cytokine release, differentiation, migration, oncosis, recombination, senescence, signal refractivity, spreading, transformation, peroxisome proliferation, necrosis, glomerulitis, nephritis, arrhythmia, hypotension, hypertension, leukemia, neutropenia renal damage, renal failure, pulmonary edema, neurotoxicity or retinopathy.

The invention further encompasses a method for identifying individuals who may be hypersensitive to the toxic side effects of drugs such as those listed in Table 1 or industrial compounds such as those listed in Table 2.

An additional embodiment includes an apparatus for identifying hypersensitivity in a subject comprising means for obtaining a gene expression profile of a number of genes associated with hypersensitivity of the subject suspected to be hypersensitive; and means for identifying in the gene expression profile of the subject a pattern of gene expression of

the genes associated with hypersensitivity, thereby to identify hypersensitivity in the subject.

Also provided are methods of determining hypersensitivity of an individual to an agent, such as a pharmaceutical drug, such as penicillin, by detecting a gene expression or protein expression profile of the individual, wherein the genes or proteins encoded therefrom are selected from genes listed in whole or in part in Tables 10 and 11. The pattern of expression may be detected in a cell, such as an immune cell, such as a leukocyte, e.g. a lymphocyte.

Also provided are devices for detection of gene expression profiles comprising nucleic acid sequences for detecting expression of the nucleic acids disclosed in the Tables, for example by hybridization. Such devices include, for example, immobilized nucleic acid arrays.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph illustrating gene expression changes associated with toxicity caused by streptozotocin.

Figure 2 is a graph illustrating co-induction of genes for hepatocyte growth factor receptor and glutathione transferase.

Figure 3 is a graph illustrating a portion of a gene expression profile from heart muscle tissue after exposure to the cardiotoxin, doxorubicin.

Figure 4 is a graph illustrating a portion of a gene expression profile from liver tissue after exposure to the peroxisome proliferation caused by WY 14,643.

Figure 5 is a graph illustrating a portion of a gene expression profile from liver tissue after exposure to the anti-neoplastic compound, carbamazapine.

Figure 6 is a chart illustrating the result of testing for penicillin hypersensitivity amongst a group of penicillin sensitive and penicillin refractive individuals by using a 180 gene penicillin array.

Figure 7 is a chart illustrating the result testing for penicillin hypersensitivity amongst a group of penicillin sensitive and penicillin refractive individuals by using a 20 gene penicillin array.

Figure 8 is a chart illustrating 20 discriminator genes analyzed for co-regulation.

Figure 9 is a graph illustrating the results of a Taqman® assay in a penicillin sensitive person.

Figure 10 is a graph illustrating the results of a Taqman® assay in a penicillin refractive person.

BRIEF DESCRIPTION OF THE TABLES

Table 1 is a list of pharmaceutical agents which potentially can cause greatly heightened toxic responses in some individuals.

Table 2 is a list of industrial agents which potentially can cause greatly heightened toxic responses in some individuals.

Table 3 is a list of genes, altered expression patterns of which can indicate and render an individual hypersensitive to drugs and chemical agents.

Table 4 is a list of genes, altered expression patterns of which can indicate and render an individual hypersensitive to drugs and chemical agents.

Table 5 is a list of genes associated with specific manifestations of organ or system toxicity.

Table 6 is a list of genes that can be associated with specific cellular manifestations of toxicity.

Table 7 lists compounds for which gene expression data in either human cells, rats or both has been generated.

Table 8 lists genes whose expression was measured when rats were exposed to the cardiotoxin doxorubicin.

Table 9 lists cell types in organs of toxicity.

Table 10 lists the characterization of genes which were isolated and sequenced from gel bands.

Table 11 lists the genes that are useful discriminator genes.

DETAILED DESCRIPTION OF THE INVENTION

Provided are methods, compositions and apparatus for identifying hypersensitivity in an individual. In one embodiment, hypersensitivity in a subject is determined by obtaining from the subject a sample from which can be determined the gene expression profile of genes associated with hypersensitivity, and identifying in the gene expression profile the presence or absence of a pattern of gene expression of the genes associated with hypersensitivity, thereby to identify hypersensitivity in the individual.

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, *Molecular Cloning: A Laboratory Manual*, second edition (Sambrook *et al.*, 1989); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Animal Cell Culture* (R.I. Freshney, ed., 1987); *Handbook of Experimental Immunology* (D.M. Weir & C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller & M.P. Calos, eds., 1987); *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis *et al.*, eds., 1994); *Current Protocols in Immunology* (J.E. Coligan *et al.*, eds., 1991); *The Immunoassay Handbook* (David Wild, ed., Stockton Press NY, 1994); *Antibodies: A*

Laboratory Manual (Harlow *et al.*, eds., 1987) and *Methods of Immunological Analysis* (R. Masseyeff, W.H. Albert, and N.A. Staines, eds., Weinheim: VCH Verlags gesellschaft mbH, 1993).

Definition of Terms

As used herein, the terms 'gene', 'polynucleotide', 'nucleotide' and 'nucleic acid' are interchangeable and refer to polynucleotide sequences, which for example, encode protein products and encompass mRNA, cDNA, single stranded DNA, double stranded DNA and fragments thereof.

The terms "protein", "polypeptide", and "peptide" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. It also may be modified naturally or by intervention; for example, disulfide bond formation, glycosylation, myristylation, acetylation, alkylation, phosphorylation or dephosphorylation. Also included within the definition are polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids) as well as other modifications known in the art.

The terms 'stress gene', 'toxicity gene' and 'toxic response gene' as used herein are interchangeable. A toxic response gene can be defined as a gene whose message or protein level is altered by adverse stimuli. The specific set of genes that cells induce is dependent upon the type of damage or toxic threat caused by the agent and which organs are most threatened. In addition to the up-regulation of genes which respond to specific toxic threat, genes which encode functions not appropriate under conditions of toxic injury may be down-regulated.

As used herein, 'toxic outcome' refers to the microscopic or macroscopic symptoms, physiological, morphological or pathological changes which are observed as a result of exposure to an agent.

A 'toxic response' as used herein refers to a cellular, tissue, organ or system level response to exposure to an agent and includes, but is not limited to, the differential expression of genes and/or proteins encompassing both the up- and down-regulation of such genes; the up- or down-regulation of genes which encode proteins associated with the repair or regulation of cell damage; or the regulation of genes which respond to the presence of an agent.

A 'gene expression profile associated with hypersensitivity' as used herein refers to the pattern of relative levels of gene expression found to be associated with hypersensitivity. Gene expression profiles may be measured in a sample, such as samples comprising a variety of cell types and may, for example, comprise blood, urine, spinal fluid or serum.

A 'protein expression profile associated with hypersensitivity' is defined as the pattern of relative levels of protein expression where said proteins are encoded by genes determined to be associated with hypersensitivity. For each gene expression profile that is determined, a corresponding 'protein expression profile associated with hypersensitivity' may be determined.

The terms 'up-regulation' and 'induction' are used interchangeably herein and refer to the regulation of gene expression, specifically the turning on of a particular gene(s). Similarly, the terms 'down-regulation' and 'repression' are used interchangeably herein and refer to the suppression of expression of a particular gene(s).

An 'agent' to which an individual is hypersensitive is defined as any substance to which an individual may be hypersensitive and includes, but is not limited to, drugs, household chemicals, industrial chemicals and other chemicals and compounds to which individuals may be exposed.

'Hypersensitivity', as used herein, refers to the exaggerated micro- or macroscopic responses of cells, tissues, organs or systems to low or average doses of an agent. These responses may lead to observable symptoms such as dizziness or nausea and can also result

in toxic outcomes. Hypersensitivity often results in toxic side effects that are different, in either degree or kind, from the response of the majority of patients at the recommended dose. Hypersensitivity may be characterized by, but is not limited to, the differential expression of genes when compared to the response of a similar individual who is not hypersensitive to a given agent. Hypersensitive individuals do not have normal gene expression patterns of key toxicologically relevant genes either prior to, or after, exposure to an agent.

'Differential expression' as used herein refers to the change in expression levels of genes, and/or proteins encoded by said genes, in cells, tissues, organs or systems upon exposure to an agent. As used herein, differential gene expression includes differential transcription and translation, as well as message stabilization. Differential gene expression encompasses both up- and down-regulation of gene expression.

The term 'individual' is used interchangeably with the term 'subject' and 'patient' and refers to a mammal, preferably the primate, more preferably the human.

The term 'normal individual' or 'normal subject' refers to individuals who exhibit the same or similar dose response curves to an agent as does the majority of the exposed population. Most drugs at high enough dosages will cause a toxic response, therefore a 'normal toxic response' refers to the toxic response elicited in an average or normal individual at high doses of an agent.

The term 'sample' as used herein refers to samples for testing or analysis. The samples may comprise cells or tissue samples and may be, for example, blood, urine or serum. Samples are characterized in a preferred embodiment by comprising at least two different genes and may also include genes from multiple cell types. Samples include, but are not limited to, those of eukaryotic, mammalian or human origin.

As used herein, "array" and "microarray" are interchangeable and refer to an arrangement of a collection of nucleotide sequences in a centralized location. Arrays can be on a solid substrate, such as a glass slide, or on a semi-solid substrate, such as

nitrocellulose membrane. The nucleotide sequences can be DNA, RNA, or any permutations thereof. The nucleotide sequences can also be partial sequences from a gene, primers, whole gene sequences, non-coding sequences, coding sequences, published sequences, known sequences, or novel sequences.

"Penicillin sensitive" refers to individuals who exhibit hypersensitivity to penicillin, for example, a higher than average immune response to penicillin. The immune response can be a hypersensitive response of any type, for example Type I, II, III, or IV. Hypersensitive reactions can include but are not limited to anaphylaxis, skin rash, and hives. Hypersensitive responses also include hypertoxicity.

"Penicillin refractive" or "penicillin insensitive" or "penicillin non-sensitive" as used herein refers to individuals who exhibit a normal or non-hypersensitive response to penicillin.

Isolating DNA/RNA from human PBL

Nucleotide sequences from human peripheral blood lymphocytes (PBL) are isolated using any number of commercially available kits i.e. from Qiagen, GenHunter, Promega, etc.

In one embodiment, total RNA is isolated from tissue samples using the following materials: Qiagen RNeasy midi kits, 2-mercaptoethanol, liquid N_2, tissue homogenizer, dry ice. It is important to take precautions to minimize the risk of RNA degradation by RNases by wearing gloves at all times and to inhibit RNase activity in work areas and equipment by treating with an RNase inhibitor such as with "RNase Zap" (Ambion® Products, Austin, TX). Autoclaving tips and microfuge tubes does not necessarily eliminate RNase enzymes and its RNA degradation activities. Samples are kept on ice when specified . Protocol which can be used is based on Qiagen® RNeasy® midi kit. This total RNA isolation technique is used for RNA isolation from human PBL and can be modified readily by one of skill in the art to accommodate different amount of human PBLs. The human PBL is

preferably taken from circulating blood of a human donor. However, human PBL can also be obtained from lymph nodes, spleen, and other tissues into which human PBLs circulate.

If tissue containing human PBL is used, then the tissue needs to be microdissected. One way is to physically break the tissue by placing it on a double layer of aluminum foil which is then placed within a weigh boat containing a small amount of liquid nitrogen. The aluminum foil is folded around the tissue and then the tissue is struck by a small foil-wrapped hammer to administer mechanical stress forces.

To preserve integrity of the RNA, all tissues are kept on dry ice when other samples are being weighed. A buffer is added to the sample to aid in the homogenization process. An example of a buffer which can be used is RLT (Qiagen®) buffer. The tissue is homogenized using any type of commercially available homogenizer (i.e. IKA Ultra Turrax T25 homogenizer, Virtishear Cyclone 750W rotor/stator homogenizer (Virtis item # 278077, etc.) can be used with the 7 mm microfine sawtooth shaft and generator (195 mm long with a processing range of 0.25 ml to 20 ml, item # 372718). After homogenization, samples are stored on ice until all samples are homogenized. The homogenized tissue sample can then be spun to remove nuclei thus reducing DNA contamination. The supernatant of the lysate is then transferred to a clean container containing an equal volume of 70% EtOH in DEPC treated H_2O and mixed. In the event that a stringy white material comes out of solution, it may then be removed. RNA is isolated by putting the supernatant through an RNeasy spin column, washed, and subsequently eluted.

In another embodiment, DNA or RNA is isolated from human PBLs obtained from a human donor. Generally, lymphocytes can be isolated from blood by separating the blood over a gradient, for example a sucrose gradient or Percoll™ or Ficoll™ gradient. Lymphocytes can be distinguished from non-lymphocyte contaminates by morphology, size and scatter by flow cytometry, or by cell surface markers such as CD2, CD3, CD4, or CD8. In general, lymphocytes which are cultured *in vitro* are non-adherent but in some instances, lymphocytes can be adherent or non-adherent depending on several factors, for

example, activation state of lymphocytes, receptors expressed on lymphocytes, and culture media contents. In some aspects, adherent cells are more problematic than non-adherent cells because of the necessity of an extra step to separate the adherent cells from the tissue culture container. However, a skilled artisan may solve this problem by treating the cells with cold PBS/EDTA solutions or an equivalent and use any number of commercially available kits, for example, from Qiagen or Ambion, to isolate the DNA or RNA from the cells. In one embodiment, total RNA of high quality and high purity can be isolated from cultured cells by using Qiagen RNeasy midi kits and 2-mercaptoethanol. This embodiment is exemplified in Example 2 *infra*. Precautions should be taken to minimize the risk of RNA degradation by RNases by wearing gloves, treating work areas and equipment with an RNase inhibitor, for example RNase Zap (Ambion® Products, Austin, TX), and keeping samples on ice. Using a Qiagen® RNeasy® midi kit (50), this total RNA isolation technique can be used for any type of cell, including but not limited to human lymphocytes and cell derived from particular organs such as kidney, liver, lung, breast, neuronal cells, skin, intestine, such as HepG2, Caco-2, MCF-7, Jurkat, Daudi, HL-60, MCL-5, SKBr-3, SKOV-3, PC-3, WISH, and HeLa.

To practice this embodiment, cells are checked under the microscope to confirm viability. Cells are then dosed with an agent, which can be a drug, chemical, or pharmaceutical composition, when they reach confluence. In a preferred embodiment, the cells are at least about 20% confluent, more preferably at least about 40% confluent, even more preferably at least about 60% confluent, and even more preferably about 80% confluent. It is preferable to avoid isolating RNA from flasks that have reached 100% confluence because the cells are no longer growing in log phase.

The adherent cells are washed and freshly prepared buffer, for example RLT buffer (RLT buffer requires the addition of 10 µl beta mercaptoethanol for each 1.0 ml RLT), is added directly to the cell culture flask. The amount of RLT buffer differs with tissue container size. Enough RLT buffer is added to cover the surface area in which the adherent

cells are growing such that most of the adherent cells come into contact with the RLT buffer. In one embodiment, T-75 flasks receive about 3 ml RLT buffer and T-175 flasks receive about 5 ml RLT buffer. It is preferable to lightly agitate the flasks at this point. Cells exposed to RLT buffer become a gelatinous layer. The cells are allowed to sit for 4 minutes, then fluid is withdrawn and is placed into and RNase-free tube. An equivalent volume of 70% ethanol is added to each tube and vortexed to distribute evenly. In the event that a precipitate with a string-like appearance forms, it is acceptable to remove and discard this string-like precipitate. The fluid is applied to a spin column, centrifuged, and the column is washed and subsequently eluted for RNA samples. The elution can be precipitated using the LiCl precipitation protocol and resuspended in RNA storage buffer for future storage. The yield can be between 200-400 μg of total RNA from a T-75 flask with greater than 50% confluency.

The isolated DNA or RNA is amplified to generate a product which can be attached to a substrate. In a preferred embodiment, the substrate is a solid substrate (i.e. glass slide). The amplification process involves using primers which have a reactive group (i.e. amine group or derivative thereof) on one end of the primer, which is incorporated into the amplification product. One example of reactive primers that can be used is Amine Primers from Synthegen. The gene fragments which are attached to the glass slide can vary in length. The more nucleotides of a gene that are in the array, the tighter the binding and the greater the specificity in binding can occur. However, it is important to consider that longer fragments are more difficult to amplify and may contain point mutations or other errors associated with amplification. Therefore, the desired length of a gene or a fragment thereof that is to be included in the array should take into consideration the balance between a high specificity of binding obtained with a long (i.e. >1 kb) gene sequence with the high mutational rate associated with a longer fragment. The gene fragments attached to the glass slide are at least about 50 base pairs (bp) in length, more preferably at least about 100 bp in length, more preferably at least about 200 bp, even more preferably at least about

300 bp, even more preferably at least about 400 bp, even more preferably at least about 500 bp in length. In a preferred embodiment, the gene fragments are about 500 bp in length. The region of a gene that is used to attach to a solid substrate to generate an array can be any portion of the gene, coding, non-coding, 5' end, 3' end, etc. In a preferred embodiment, about 500 base pairs of the 3' end of canine gene related to toxicological responses are selected to be included in an array.

Several techniques are well-known to a skilled artisan for attaching a gene or a fragment thereof to a solid substrate such as a glass slide. One method is to attach an amine group, a derivative of an amine group, another group with a positive charge or another group which is reactive to one end of a primer that is used to amplify a gene or a gene fragment to be included in the array. Subsequent amplification of a PCR product will then incorporate this reactive group onto one end of the product. The amplified product is then contacted with a solid substrate, such as a glass slide, which is coated with an aldehyde or another reactive group which will form a covalent link with the reactive group that is on the amplified PCR product and become covalently attached to the glass slide. Other methods using amino propryl silicane surface chemistry are disclosed by Corning Company at <http://www.cmt.corning.com.> other methods for making microarrays which are readily accessible at <http://cmgm.stanford.ecu/pbrownl>

In one embodiment of the invention, fluorescence-labeled single strand (or "first strand") cDNA probe is made from total or mRNA by first isolating RNA from control and treated cells, disclosed *supra*. This probe is hybridized to microarray slides spotted with DNA specific for toxicologically relevant genes. This is exemplified in Example 8-14. The materials needed to practice this embodiment are: total or messenger RNA, primer, Superscript II buffer, dithiothreitol (DTT), nucleotide mix, Cy3 or Cy5 dye, Superscript II (RT), ammonium acetate, 70% EtOH, PCR machine, and ice. The Cy™ dyes may be obtained from Amersham. The embodiment may also be practiced with equivalents of the

materials listed above, for example, SuperScript II may be replaced with an equivalent enzyme and Cy5 and Cy3 may be replaced with another fluorescent dye.

In one embodiment, a discrete amount of RNA, for example 20μg of total RNA or 2μg of mRNA, is used to generate cDNA. In a preferred embodiment, the volume is no more than 14 μl. If RNA is too dilute, the samples are concentrated to a volume of less than 14 μl in a centrifuge with vacuum (i.e. Speedvac) without heat. The Speedvac should be capable of generating a vacuum of 0 Milli-Torr so that samples can freeze dry under these conditions. It is preferable for the tubes containing RNA to be kept on ice to avoid RNA degradation until the next step is ready to proceed. Following standard techniques well-known in the art, cDNA samples are amplified from RNA templates. A mixture of fluorescent dyes is made for labeling the cDNA samples. A variety of dyes can be used. In one embodiment, Cy3 dye, which is pink-red, and Cy5 dye, which is blue, are used. The Cy dyes are light sensitive, therefore, any solutions or samples containing Cy-dyes should be kept out of light, i.e. cover with foil. Example 9-12 discloses preferred mixtures and methods of using Cy3 and Cy5 dyes for labeling cDNA samples and purification steps therewith.

In an embodiment wherein the sequences of toxicologically relevant genes are not known and canine cells are divided into two groups, untreated and treated, to identify toxicologically relevant genes as disclosed *infra*, Cy3 dye mixture is incubated with the cDNA of each treated sample and Cy5 dye mixture is incubated with the cDNA of each control sample. Following the methods disclosed in Example 9, a visible pellet can be seen which is pink/red for cDNA incubated with Cy3 and blue for cDNA incubated with Cy5. It is recommended that the tubes are centrifuged at a fixed position so the pellet will be at a known area in the tube. In some rare instances, the cDNA sample (or cDNA probe) is seen spread on one side of the tube instead of a tight pellet. If the pellet is white (no pink/red or blue), it is likely that the reaction has not occurred to maximal efficiency.

Purification of fluorescent probes

Purification of fluorescence-labeled first strand cDNA probes can be achieved in one embodiment using the following materials: Millipore MAHV N45 96 well plate, v-bottom 96 well plate (Costar), Wizard DNA binding Resin, wide orifice pipette tips for 200 to 300 µl volumes, isopropanol, and nanopure water. It will be evident to a skilled artisan that equivalent products may be substituted in other embodiments, i.e. other types of tissue culture plates, binding resin from other commercially available sources, for example Qiagen. Plate alignment during centrifugation is important since misaligned plates can lead to sample cross contamination and/or sample loss. In one embodiment, probes are purified by binding to a resin. The binding resin can be obtained by itself or from a kit provided by any number of commercial sources, i.e. Qiagen, Promega, etc.

Fluorescence Readings of cDNA Probe

Incorporation of fluorescence into cDNA probes can be achieved by using a number of methods. In one embodiment, the following material is used: 384 well, 100 µl assay plate (Falcon Microtest cat#35-3980) and Wallac Victor 1420 Multilabel counter (or equivalent). Prior to use as a cDNA probe in hybridization, cDNA probes are purified and concentrated as exemplified in Example 10.

It is preferable that a consistent amount of cDNA is pipeted into the plate wells because readings can vary with volume. Controls or identical samples can be pooled at this step, if required or desired. The Cy-3 and Cy-5 fluorescence are analyzed using a fluorimeter, luminometer, flow cytometer, or any equivalent device which can detect different fluorescent dyes at different wavelengths. In a preferred embodiment, the Wallac 1420 workstation programmed for reading Cy3-Cy-5 is used. A typical range for Cy-3 (20µg) is 250-700,000 fluorescence units. A typical range for Cy-5 (20µg) is 100-250,000 fluorescence units. Preferred settings for the Wallac 1420 fluorescence analyzer are as follows:

Cy3
CW lamp energy = 30445
Lamp filter = P550 slot B3
Emission filter= D572 dysprosium slot A4
Emission aperture = normal
Count time = 0.1 s

Cy5
CW lamp energy = 30445
Lamp filter = D642 samarium slot B7
Emission filter= D670 slot A8
Emission aperture = normal
Count time = 0.1 s

After detection of the cDNA probes, it is important to concentrate the cDNA probes so that they can be resuspended in hybridization buffer at an appropriate volume for hybridizing to the array. Internal normalization is achieved by taking into consideration the ratio of Cy5 fluorescence to Cy3 fluorescence in the treated and untreated canine cell groups, respectively.

Microarray Hybridization

Hybridization of labeled cDNA probes to single stranded, covalently bound DNA target genes on glass slide microarrays can be accomplished by a variety of methods. In one embodiment, exemplified in Example 7, the following material are used: formamide, SSC, SDS, 2 μm syringe filter, salmon sperm DNA, hybridization chambers, incubator, coverslips, parafilm, and heat blocks. It is preferable that the array is completely covered to ensure proper hybridization. Hybridization buffer is prepared with consideration towards stringency. Stringency can be varied by increasing or decreasing the amount of SSC and detergent (i.e. SDS, Triton, etc.). Stringency can also be varied by the temperature at which the hybridization occurs. A higher temperature tends towards high stringency conditions. A skilled artisan can determine, in a stepwise fashion, the stringency of the hybridization buffer desired. Clean slides and coverslips are desirable and can be

obtained using N_2 stream. Hybridization buffer is added to dried probe and mixed in the dark at room temperature and then brought to a higher temperature in a heat block. Each probe can remain in a heat block until it is ready for hybridization. The probe is applied to a slide or to a coverslip and then covered with slide. It is highly preferable to avoid the material at the bottom of the tube and to avoid generating air bubbles. This may mean leaving some residual volume in the pipette tip. Slides are then placed in a hybridization chamber, wrapped to prevent the liquids from desiccating. One problem that can occur with overly dried slides is increased fluorescence on the edge of the spot containing the target gene fragment to which the labeled cDNA probe binds. In an alternative, the hybridization chamber can have a built-in humidity gauge to avoid desiccation of the slides. In a preferred embodiment, the slides are placed in a 42°C humidity chamber in a 42°C incubator for 18 to 24 hours. It is preferable to avoid probes or slides sitting at room temperature for long periods.

Post-Hybridization Washing

To obtain single stranded cDNA probes on the array, all non-specifically bound cDNA probe should be removed from the array. In one embodiment, removal of all non-specifically bound cDNA probe can be accomplished by washing the array using the following materials: slide holder, glass washing dish, SSC, SDS, and nanopure water. Equivalents of SSC and SDS may also be used as substitutes. It is highly preferable that great caution be used with the standard wash conditions since deviations can affect data significantly.

In one embodiment, glass buffer chambers and glass slide holders are filled with heated SSC buffer with sufficient volume to submerge the microarrays. It is important to exercise caution in heating of the SSC buffer since a high temperature may strip off the probes, preferably the temperature is at most about 60°C, more preferably at most about 50°C, even more preferably at most about 40°C, and even more preferably at most about

35°C. A skilled artisan can vary the concentration of SSC in the buffer according to the stringency desired. The slides are placed in buffer which may contain SSC and/or detergent (i.e. SDS, Triton, etc.) and the coverslips are dislodged and fall off the slide within several minutes of submersion. In the event that the coverslips do not fall off within several minutes of submersion, very gentle agitation may be administered to the chamber in which the wash is being conducted to dislodge the coverslips. The slides with the hybridized probes are subjected to several rounds of washes with different conditions. In one embodiment, a detergent (i.e. SDS) is added to the wash buffer in different concentrations and the slides are washed in this buffer before a final wash in nanopure water. The slides are dried in a manner that will minimize background signal of the array. A preferred method of drying is to use a folded paper towel underneath the slide and a gently dabbing motion on the slide with a tissue. It is important that the slides do not air dry since this will lead to increased background.

Gene Expression Profiles

The pattern of gene expression characteristic of hypersensitivity is predetermined, and is, for example, provided in a database. By comparing the gene expression profile of the subject with the predetermined pattern of gene expression of multiple genes characteristic of hypersensitivity, the hypersensitivity of the subject can be conveniently and rapidly determined. Advantageously, the invention provides a large number of predetermined gene expression patterns of genes associated with hypersensitivity, for example in a database, so that a large number of genes can be rapidly analyzed and compared in the subject. Analysis of information about expression of a wide spectrum of genes associated with hypersensitivity facilitates the rapid determination of hypersensitivity of a subject to an agent, or multiple agents.

For example, the differential gene expression profile associated with a given agent can be determined for a given agent using, for instance, eukaryotic or mammalian cells or

cell lines or animal models and exposing a population of the eukaryotic or mammalian cells or cell lines or animal models to an agent and comparing their gene expression to the same type of eukaryotic or mammalian cells or cell lines or animal models from an untreated population to determine the gene expression profile associated with hypersensitivity.

Hypersensitivity to an agent, for example, a pharmaceutical drug or household, industrial or other chemical, can be rapidly determined with samples from an individual or group of individuals by treating the sample(s) with an agent and comparing the gene expression profile with the gene expression profile associated with hypersensitivity determined previously for a particular agent and, for instance, stored in a database and accessed and compared with associated software.

Table 1 lists approximately 200 drugs sold in the U.S. and Europe. There are individuals who are hypersensitive to the toxic side effects of each of these drugs. Table 2 lists at least 100 major industrial chemicals for which there is documented evidence of toxicity due to occupational exposure. For each of these chemicals there are individuals whose toxic response is heightened compared to the majority of the population.

In a preferred embodiment, multiple genes are analyzed. Preferably, the number of genes, associated with hypersensitivity, whose expression levels are determined and which comprise the gene expression profile is large; for example, one or more, at least 2, at least 3, at least 4, at least 5, at least 10, at least 50, at least 100, or at least 250. The present invention also encompasses gene expression profiles where the number of genes is greater than 400, 500, 600 or more.

In another embodiment, the genes, whose expression levels comprise the gene expression profile, are drawn from a variety of cell types.

For example, the genes, whose expression levels comprise the gene expression profile, are drawn from cells of a number of different tissues or organs.

In another embodiment, cells or tissues derived from an individual are used to establish primary cell cultures, for example fibroblasts, hepatocytes, and other examples

known in the art. These primary cell cultures are then exposed to the agent. Cell cultures established from the appropriate tissues of hypersensitive individuals are more sensitive to the toxic effects of the agent than cultures established from normal individuals. This hypersensitivity is reflected in the gene expression patterns elicited from the cell cultures.

In another embodiment, cells or tissues derived from an individual are used to establish primary cell cultures, for example fibroblasts, hepatocytes, and other examples known in the art. Co-cultures would be grown from two or more cell types that reflect the cell types involved in systemic toxicity. These co-cultures are then exposed to the agent of interest. Cell co-cultures established from the appropriate tissues of hypersensitive individuals are more sensitive to the toxic effects of the compound than co-cultures established from normal individuals. This hypersensitivity is reflected in the gene expression patterns elicited from the cell co-cultures.

In another embodiment, the gene expression profile consisting of the expression levels of multiple genes includes genes drawn from a single cell, tissue or organ type, and the profile is examined to determine the association of the gene expression profile with hypersensitivity.

In addition to the determination of absolute levels of expression for the genes in the gene expression profile associated with hypersensitivity, the relative expression levels of two or more genes in the gene expression profile associated with hypersensitivity can be determined and can be relevant to a determination of hypersensitivity. Hypersensitive individuals will have profiles of expression of relevant toxicity genes that are distinct from individuals who are not hypersensitive.

In another embodiment, gene expression profiles from normal individuals, hypersensitive individuals or cell cultures are established for individual agents to determine possible toxic drug-drug interactions when patients (normal or hypersensitive individuals) are treated with multiple drugs. There are hundreds of combinations of compounds that are more toxic when taken together than when taken singly. Usually these toxic drug-drug

interactions are discovered as clinical manifestations once the drugs reach market. Examples of compounds that cause severe toxicity when taken together include cyclosporin A and trimethoprim, Walworth *et al.* (Lancet) 1:336(1983); and Clonidine and Tricyclic antidepressants such as Amoxapine. Briant *et al.* (Br J Pharmacol) 46:563(1972). The expression of the same pattern of toxic response genes for two or more compounds in either normal or hypersensitive individuals, indicates that the two or more compounds, taken together, will often show a synergistic toxic effect. Gene expression profiles for each compound, determined *in vitro* or *in vivo*, allows prediction of the severe toxicity if the two compounds were taken together.

In another embodiment, the gene expression profile of genes associated with certain disease states is analyzed. Normal individuals can become temporarily hypersensitive to the toxicity of certain drugs because of disease states. Hypersensitivity is present in normal individuals when toxic defense mechanisms are temporarily compromised. For example, an individual who suffers from AIDS-induced immunosuppression will be hypersensitive to the toxic effects of immunosuppressive compounds such as cyclosporin A. An individual suffering from pulmonary edema due to viral infection will be temporarily hypersensitive to compounds such as bleomycin which elicit pulmonary edema as a toxic side-effect.

In another embodiment, the method includes obtaining a protein expression profile of a number of proteins encoded by genes of the subject, determining if the protein expression profile of the subject comprises a pattern of protein expression associated with hypersensitivity to an agent, and withholding the agent from those individuals or altering the therapy or dosage and closely monitoring the individual for toxic effects.

In addition, a method of identifying a number of genes associated with hypersensitivity to an agent is provided comprising comparing the protein expression profile, where the proteins are encoded by the genes identified as associated with hypersensitivity to the agent, of cells treated with the agent with the protein expression

profile of cells not treated with the agent and determining proteins that have altered expression due to the exposure to the agent in the treated cells. The cells may comprise, for example, a variety of different cell types and each cell type may comprise a gene associated with hypersensitivity to the agent, and the protein encoded by gene.

An additional embodiment includes a method of identifying a number of genes associated with hypersensitivity to an agent which comprises comparing the protein expression profile, where the proteins are encoded by the genes identified as associated with hypersensitivity to the agent, of cells treated with the agent with the protein expression profile of the same type of cells from the same subject not treated with the agent and determining proteins that have altered expression due to exposure to said agent in the treated cells. The cells may comprise, for example, a variety of different cell types and each cell type may comprise a gene associated with hypersensitivity to the agent, and the protein encoded by the gene.

In a further embodiment, the gene expression profile of multiple genes associated with cellular response to toxic agents are analyzed to determine the association with hypersensitivity of the genes in the profile.

Using the methods, compositions and devices disclosed herein, rapid, accurate and inexpensive tests of an individual can be conducted in order to confirm whether the individual is hypersensitive to an agent. For example, an individual can be screened for hypersensitivity to a drug before the drug is administered. Such screenings avoid incidents of hypersensitivity in individuals to whom a drug might otherwise be administered. Alternately, the drug can be given in lower doses to hypersensitive individuals and/or those individuals considered at risk may be closely monitored for adverse reactions to the agent. Avoiding exposing hypersensitive individuals to any given drug or compound, or to a higher than necessary dose or level of the drug or compound, provides cost savings to manufacturers who may produce the drug or compound with an assurance that hypersensitivity reactions will be avoided. Those who are not hypersensitive may safely

receive the drug or compound and receive its benefits, while those who are hypersensitive may safely avoid the drug or be prescribed a different drug or in the case where the toxicity is due to exaggerated pharmacological effects, a smaller, but just as effective dose.

The invention also encompasses using the methods, composition and devices disclosed herein for rapid, accurate and inexpensive tests that can be used, for instance, to determine the causative agent in an individual exhibiting symptoms consistent with or indicative of a toxic response or hypersensitivity to various agents. By ascertaining the gene profile of a number of genes associated with particular cells, tissues, organs or systems, the agent eliciting the toxic response or hypersensitivity may be determined and thereon avoided. In one embodiment, gene expression analysis might be used to determine the nature of the toxic insult and thus provide treatment. For example, analysis of expression of tox-response genes might aid in the effective diagnosis and treatment of an unconscious child suspected of having been inappropriately exposed to a drug or chemical agent. Gene expression patterns could be useful in determining if the unconscious state were the result of exposure to a soporific agent or one that inhibited mitochondrial function, the treatments of which would be quite distinct.

Exemplary genes associated with hypersensitivity whose expression may be screened in order to determine hypersensitivity are provided in whole or in part in Tables 3, 4, 5, 6, 8, 10 and 11. Also provided herein are methods of identifying genes associated with hypersensitivity.

Genes

Tables 3, 4, 5, 6, 8, 10 and 11 provide a list of exemplary genes from which genes associated with hypersensitivity to a particular agent may be selected. Genes selected from Table 3 and Table 4 are responsive to toxic stimuli and important to the defense or repair of toxic damage. Individuals with significantly altered expression levels of two or more of the genes in Tables 3, 4, 5, 6, 8, 10 and 11 can also show different toxic responses from normal

individuals. For a given agent, the expression profile of two or more genes, for example, selected from Tables 3, 4, 5, 6, 8, 10 and 11 can be obtained from a cell, tissue or organ and, a pattern of gene expression predetermined to be associated with hypersensitivity can be established.

Genes such as those selected from Tables 3 and 4 are evaluated for differential gene expression, for example in the major toxic target organs in humans and/or rats and mice. Examples of genes in which differential expression is indicative of toxicity or hypersensitivity in specific organs or systems such as liver (hepatic), kidney (renal), lung (pulmonary), central nervous system (neural), heart (cardio) and immune system are shown in Table 5.

As an example, Figure 1 shows the pattern of gene expression of approximately 250 genes in the liver when the subject received a relatively high dose of streptozotocin. Samples, including for instance, blood, urine, serum or tissue, from individuals known to be hypersensitive to streptozotocin can be obtained after the subject is treated with streptozotocin. Alternately, for example, samples may be from untreated individuals known to be hypersensitive to streptozotocin and the samples may then be treated *in vitro* with streptozotocin. The samples are then examined to identify genes associated with hypersensitivity. This may show, for example, highly exaggerated expression of toxic response genes and/or patterns of induction or repression of genes in treated individuals or upon *in vitro* treatment of the sample with streptozotocin compared to individuals who are not hypersensitive or sample which is not treated with streptozotocin. As streptozotocin is an example of a bulky alkylating agent, individuals who are hypersensitive to streptozotocin may be tested for hypersensitivity to compounds with similar toxic properties, such as bulky alkylating agents, such as merbarone and carmustine.

Genes whose levels of expression change in response to toxic stimuli may be evaluated. Examples of genes with expression changes in response to toxic stimuli are listed in Tables 3 and 4. The genes in Table 3 and Table 4 have been shown to be induced

in either cell lines, primary cells, tissues or tissue slices, from human or animal origin. For example, the GADD 153 gene has been shown to be induced in many human cell lines upon exposure to radiation. The environmentally important compound trichloroethylene was recently demonstrated to cause induction of several genes, including c-Myc and c-Jun in mice exposed to low toxic levels for 24 hr. Tao et al.(J Biochem Mol Toxicol) 13(5): 231-7 (1999). In primates, closely related to humans, hyperoxia causes increased expression of the genes encoding thioredoxin and thioredoxin reductase gene expression in lungs. Das et al., (Chest) 116(1 Suppl): 101S (1999).

Many of the genes in Tables 3 and 4 are known to be involved in the prevention or repair of damage to DNA, cells or tissue in response to toxic agents (several examples are provided by the following references: Kegelmeyer et al. (Mol. Carcinog.) 20(3): 288-97 (1997); Koerber et al. (Mol. Reprod. Dev.) 49(4): 394-9 (1998); Kuhn (Nutr. Rev.) 56:11-9, discussion 54-75 (1998); Lu et al. (Mol. Carcinog.) 20(2): 204-15 (1997); Muhlenkamp et al. (Toxicol. Appl. Pharmacol.) 148(1):101-8 (1998); Melhus et al. (Biochem. Mol. Biol. Int.) 43(5):1145-50 (1997); Pentecost (Steroid Biochem. Mol. Biol.) 64(1-2):25-33 (1998); Quattrochi et al. (Arch. Biochem. Biophys.) 349(2):251-60 (1998); Rout et al. (Cell Calcium) 22(6): 463-74 (1997); Sadekova. et al (Int. J. Radiat. Biol.) 72(6): 653-60 (1997); Yuan et al. (J. Biol. Chem.) 273(7):3799-802 (1998); Zhao et al. (Oncogene) 16 (3):409-15 (1998).

Table 6 shows a set of genes associated with specific types of cellular toxicity. Studies of single gene expression have shown over- or under- expression of certain of these genes, to affect the sensitivity of the cell or organism to toxic stimuli and are described in the art. Advantageously, the expression levels of all of these genes can be measured simultaneously. Individuals hypersensitive to an agent can be identified by measuring the expression patterns of the toxicity genes specific to that agent. Tables 3, 4, 5 and 6 are non-limiting examples of such toxicity genes.

Agents

Many compounds are toxic at a high enough concentration. For example, while most individuals might experience extreme tachycardia after receiving a very high dose – 20 times normal - of a drug, they experience no such effects at recommended doses. The hypersensitive individual would experience extreme tachycardia at the recommended dose or at a lower than normal dose. A hypersensitive individual might also experience a qualitatively distinct toxic response to a compound, not just the same response that a normal individual would experience at high doses. For example, the hypersensitive patient might experience extreme dizziness, a side effect not reported by individuals even at high doses.

Agents to which individuals may be hypersensitive, and for which hypersensitivity can be determined, may include, for example, drugs, industrial chemicals, household or other chemicals, including those in the workplace. Examples of drugs and industrial chemicals for which a sub-population is hypersensitive are listed in Tables 1 and 2.

As a further example, individuals who are employed in manufacturing or other environments which expose them to a variety of agents may be screened for agents to which they might come into contact. Individuals, or for example, a subset of workers, who are hypersensitive to the agents can then be identified. Hypersensitivity to other agents also may also be determined, such agents including, but not limited to biological agents such as naturally occurring organic compounds, including proteins, saccharides and lipids.

Exemplary pharmaceutical agents include, for example, tienilic acid, halothane, dihydrazine, diclofenac, fialuridine, carbamazepine, Trovan™ (trovafloxacin), Seldane™ (terfenadine), hismanol, dihydrolazine, warfarin, phenytoin, omeprazole, diazepam, haloperidol, perphenazine, perhexiline, phenformin, tolbumamide, penicillin, clozapine, aminopurine, quinidine and remoxipide. Table 1 lists additional agents for which there are individuals who demonstrate hypersensitivity.

Examples of other chemicals include industrial chemicals, such as paint, volatile organic compounds (VOCs), solvents, adhesives, pesticides, herbicides, perfumes, aerosols, cleaning compounds and synthetic polymers such as textiles.

Identification of Genes

Genes initially suspected of being associated with hypersensitivity and hence potentially useful in the present invention are identified, for example, by conducting extensive literature searches; investigating known biochemical pathways with toxicological relevance; and measuring gene expression from toxin-exposed animals, humans or cell lines. Hypersensitivity to an agent, such as a drug, may also be determined based on the ability to identify the underlying molecular basis for the toxicity of specific drugs. Hypersensitivity can also be determined by examining the gene expression of hypersensitive and normal individuals.

In one embodiment, methods are provided wherein literature reports on the expression levels of single genes in response to a single agent are collected, for example, in a database, and then analyzed to establish patterns of expression that can be correlated to hypersensitivity. Advantageously, large amounts of data can be collected and analyzed, for example by software means. For example, Matrix Express™, and Chem Profiler™ (Phase-1 Molecular Toxicology, Santa Fe, NM) accommodate capture and analysis of gene expression profiles. For example, it allows identification of induced genes from the total set of genes measured using a number of criteria; for example, statistical significance, two-fold, and 1.5 X the standard deviation. The software also allows the search of other profiles and determines the commonality between subsets, ranking profiles by several measures of similarity, for example, using all or a subset of the genes.

Experiments include both *in vivo* and *in vitro* responses to agents, for example, the exposure of eukaryotic, mammalian or human cells, and animals to agents listed in Table 7.

One ultimate benefit of this exercise is to reduce the need for animal testing. Each agent is tested at several concentrations and time points.

The toxicology of an agent is evaluated by measuring toxic insult by detecting observable changes in organ or system appearance and/or function, at the micro- or macroscopic levels. For example, a drug may cause changes in fatty acid metabolism in liver hepatocytes. This in turn causes observable changes in liver appearance, such as a specific toxicological outcome referred to as fatty liver. In order for cells, and thus tissues and organs, to undergo observable morphological changes due to toxic insult, they generally express a subset of genes differently than untreated cells. Thus, manifestations of toxic injury frequently require differential gene expression. Such genes that are differentially expressed in response to toxic injury are evaluated for use as genes associated with hypersensitivity in accordance with the present invention.

Thus, the expression of genes that are differentially expressed in total across cell, organ and tissue types in humans, in particular in response to toxic insult, may be evaluated to determine which genes have expression that is linked to hypersensitivity in an individual. Individuals who do not properly express the appropriate toxicity response genes for a specific compound will be hypersensitive to the toxic effects of that compound.

Organs are composed of tissues, which in turn are composed of various cell types. There is a core set of genes whose products are involved in functions essential to all cells, and whose expression is shared by most human cell types. In addition to these common core genes, each cell type expresses a set of genes that is unique to that cell type. When animals, including humans, are exposed to chemicals that cause damage to one or more organs, cells that comprise those organs attempt to mitigate or repair that damage by turning on genes that encode toxic-damage defense or repair proteins. The specific set of genes that cells induce is dependent upon the type of damage or toxic threat caused by the compound and upon which organs are most threatened. In addition to the genes that are induced to deal with the specific toxic threat, there may be genes which encode functions

that are not needed nor appropriate under conditions of toxic injury. Therefore, both the up- and down-regulation of genes can be measured in order to understand the molecular response to that compound, and the linkage of gene expression to hypersensitivity. The pattern of differential gene expression within the toxic target organs can be limited to a relatively small number of genes, and may be very specific to both the organ being threatened and the type of damage. Such genes may be analyzed to determine which genes are responsible for hypersensitivity, for example, within a certain organ. Such genes may be analyzed to identify subsets of genes that are associated with hypersensitivity to certain agents.

The measurement of gene expression patterns is useful because many factors can affect the level of transcripts of toxicity genes, including mutations in the regulatory regions of genes, mutation in transcription factor that control the gene(s) of interest, and gene duplications and deletions. Examples of genes whose expression may be screened for association with hypersensitivity to certain agents are further discussed herein.

Genes associated with changes in expression levels due to adverse stimuli or toxic insult include, for example, genes which respond to the presence of a compound, and genes which respond to damage caused by a compound at, for example, the protein, nucleotide, macromolecular, membrane, cell, tissue, organ or system level. For example, certain proteins either prevent or repair toxic cellular injury. Individuals who do not express the appropriate gene profile will suffer greater damage from toxic compounds through a lack of repair enzymes.

Toxic responses can be measured by pathological changes, for example, at the protein, nucleotide, cell, tissue, organ or system level. These pathological changes can be associated with differential gene expression of at least two genes. In addition, and the correspondence between the pathological change and the differential gene expression can be established. At the concentration where pathological outcomes are observable, gene expression changes are specific and causally related to the outcome. For example,

compounds that cause peroxisome proliferation as observed in the electron microscope, such as WY 14,643 (Sigma Chemicals; St. Louis, MO), a common toxicological compound known in the art, turn on genes causally related to peroxisome proliferation (See Figure 4). Compounds that cause DNA damage as manifested by increased mutations and cell-cycle disruption turn on genes required to alter the cell cycle and repair the damage (See Figure 5 below). Furthermore, since most drugs elicit pleiotropic effects, and are metabolized differently, there is a specific gene expression pattern for each compound, even though there may be a sub-pattern with all compounds that, for example, alkylate DNA at the O-4 position of thymine.

Genes associated with hypersensitivity also may be identified by examination of the gene expression profile of hypersensitive individuals differing from normal gene expression patterns of the genes associated with differential gene expression either before or after exposure to the particular drug in question.

Genes which may be identified and tested for their association with hypersensitivity to a certain agent include a variety of genes known in the art that are induced in mammalian or eukaryotic cells or cell lines exposed to high concentrations of chemicals. Genes associated with toxicological response that can be identified for predicting different types of hypersensitivity to different agents include, for example, those genes described in: Cattell (Semin. Nephro.) 19(3):277-87 (1999); Schnabel, M. et al. (Int. J. Mol. Med.) 1(3):593-5 (1998); Cruse et al. (Carcinogenesis) 20(5) 817-824 (1999); Fogg, S. et al. (Am. J. Respir. Cell Mol. Biol.) 20(4):797-804 (1999); Aoki et al. (FEBS Lett.) 333:114-118 (1993); Feuerstein et al. (Can. J. Physiol. Pharmacol.) 75(6):731-4 (1997); Rodrigo et al. (Scand. J. Gastroenterol.) 34(3):303-307 (1999); Schmidt et al. (Biochem. Biophys. Res. Commun.) 242: 529-533 (1996); Rockett et al. (Eur. J. Drug Metab. Pharmacokinet.) 22: 239-233 (1997); Rudat et al. (Int. J. Radiol. Bio.) 73: 325-330 (1998); Buters et al. (Proc. Natl Acad. Sci USA) 96(5): 1977-1982 (1999); Wang et al. (Cardiovasc. Res.) 35

:414-421 (1997); Pang et al.(Ann. Hum. Genet.) 62(3): 271-4 (1998); and Herrlich, et al. (Biol. Chem.) 378(11) :1217-29 (1997).

Many toxic response genes are induced to higher levels of expression only when needed. An individual can show a defective or hypersensitive response if a crucial protein is defective or is not produced in sufficient abundance when needed. Thus individuals who do not synthesize sufficient amounts of key proteins or produce defective proteins required to minimize the toxic damage from a given agent will suffer from greater toxic injury.

Altered levels of the gene products of the genes listed in Table 3, Table 4, Table 5 and Table 6 are likely to render the cell or organism hypersensitive to toxic stimuli, and there is great variability among the population in basal and induced levels of these genes. There have been many studies of some of these individual genes in the literature, some of which are discussed below. For example, a mouse knock-out mutant for the DNA repair gene PARP was shown to be hypersensitive to the toxicity and genetic damage caused by gamma-irradiation and MNU. Trucco C. et al. (Mol Cell Biochem) 193(1-2): 53-60 (1999). Humans with low basal or induced expression of the PARP gene will be hypersensitive to gamma-irradiation, MNU and all radiomimetic agents.

In another example, it was recently shown that DNA repair methyltransferase (Mgmt) knockout mice are hypersensitive to the toxic effects of several chemotherapeutic alkylating agents. Glassner et al. (Mutagenesis) 14(3): 339-47 (1999). Individuals with decreased expression of the Mgmt gene will be hypersensitive to the same compounds.

In another example, a 'temporary' knock-out of the cyclophilin-A gene in mice was made by injecting an anti-sense RNA against the cyclophilin A gene in rat neonatal cardiomyocytes. The expression level of the cyclophilin A gene was reduced by 93% and animals treated were hypersensitive to the toxic effects of t-butylhydroperoxide. Doyle et al. (Biochem. J.) 341(1):127-32 (1999). Humans who show depressed levels of cyclophilin A gene expression are expected to be hypersensitive to the toxic effects of t-butylhydroperoxide and other compounds that form active oxygen radicals.

Polymorphisms occur in the human population for the gene encoding serum paraoxonase (PON1). The PON1 gene product plays a major role in the detoxification of organophosphate (OP) compounds. One polymorphism (Arg192 isoform) hydrolyzes diazoxon, soman and sarin slowly. Costa et al. (Chem. Biol. Interact) 119-120: 429-38 (1999).

Genes associated with hypersensitivity can be selected from those in Table 3, which are induced by toxic damage and have important physiological roles in responding to toxic stimuli. For example, Rettie et al. (Epilepsy Res.) 35(3):253 (1999) showed that humans carrying a polymorphism that decreases expression of the CYP2C9 gene are very sensitive to compounds such as phenytoin and (S)-warfarin. The data demonstrate that the CYP2C9*3 polymorphism gene product retains only 4-6% of the metabolic efficiency of the wild-type protein CYP2C9*1 towards phenytoin and (S)-warfarin. Individuals who show dramatically reduced expression of the normal CYP2C9*1 could show the same hypersensitivity to these drugs.

Several factors can affect the basal and induced levels of expression of these genes. For example, mutations or polymorphisms that affect the promoter region of tox-response genes can cause hypersensitivity to compounds. For example, several polymorphisms have been identified in the promoter region of the human HLA-DQA1 gene that affect the levels of mRNA and thus protein levels of the HLA haplotype. Indovina, P. et al. (Hum. Immunol.) 59(12): 758-67 (1998). Polymorphisms in the regulatory region of the genes encoding plasminogen activating inhibitor increase the risk for developing coronary heart disease (Grenett et al. (Arterioscler. Thromb. Vasc. Biol.) 19(11):1803-1809 (1998). The polymorphisms mentioned above for human plasminogen activating inhibitor are in the regulatory region of the gene and result in altered expression of the gene. This risk of developing coronary heart disease, and likely increased risk to drugs with cardiotoxic properties, is increased specifically as a function of the altered expression levels. Many toxic stimuli induce or repress TGF-β1 levels. Individuals who overexpress

TGF-β1 show heightened levels of apoptosis and fibrosis seen with mycotoxin-induced liver injury. Cruse et al. (Carcinogenesis) 20(5):817-824 (1999).

Polymorphisms in the gene encoding the vitamin D receptor change differential expression of many downstream genes and render the individual likely to develop drug induced psoriasis. Park et al. (Arterioscler Thromb Vasc Biol) 19(11):1803-1809 (1999). Expression levels of the gene for cytochrome P450 CYP1B1 have a strong effect on the susceptibility to 7, 12-dimethylbenz[a]anthracene-induced lymphomas. Thus individuals who do not express appropriate levels of the P450 CYP1B1 gene would be at enhanced risk for toxic side effects of compounds like 7, 12-dimethylbenz[a]anthracene that are metabolized by that P450 protein. Butlers et al. (Proc. Natl. Acad. Sci. USA) 96(5):1977-1982 (1999). Classic quotidian fever was found to be associated with significantly lower levels of plasma IL-6. The published evidence shows that there is a genetically determined difference in the degree of the IL-6 response to stressful stimuli between individuals (Coulthard et al. (Blood) 92(8): 2856-62 (1998). Thus individuals with genetically linked quotidian fever are likely to be at enhanced risk for a number of drugs that elicit IL-6 expression as part of their inherent toxicity.

The level of expression of the enzyme thiopurine methyltransferase is an important determinant of the metabolism of thiopurines used in the treatment of acute lymphoblastic leukemia and acute myeloid leukemia. TPMT expression displays genetic polymorphism with 10% of individuals having intermediate and one in 300 undetectable levels. Individuals who do not express TPMT are at extreme risk of severe cardiotoxicity when treated with compounds such as azathioprine (Collie-Duguid et al. (Pharmacogenetics) 9(1):37-42 (1999); Coulthard. et al. (Blood) 92(8):2856-62 (1998). In another example where altered expression of tox-response genes affects the response to specific drugs, experiments have recently demonstrated that overexpression of the human HAP1 protein sensitizes cells to the lethal effect of bioreductive drugs. Prieto-Alamo et al. (Carcinogenesis) 20(3):415-9 (1999).

Altered expression can come from many causes besides mutations in the promoter region. These include, include mutations in the transcription factors or receptors that regulate a gene and gene duplications. While cDNA sequence analysis of a normal sequence that had been duplicated would not detect any change in the coding regions of the genes of interest, gene expression analysis would. For example, two active copies of the X-linked gene spermidine/spermine N1-acetyltransferase (SSAT) in a female lung cancer cell line have been associated with an increase in sensitivity to an anti-tumor polyamine analogue. Mank-Seymour et al. (Clin. Cancer Res.) 4(8): 2003-8 (1998). Duplications in the CYP2D6 or CYP2C19 genes have been shown to be linked with sensitivity to a number of drugs including warfarin, codeine and clofenac. Lundqvist et al. (Gene) 226 (2): 327-38 (1999); Yasar et al. (Biochem. Biophys. Res. Commun.) 254 (3): 628-31 (1999).

There are numerous examples where expression polymorphisms comprise a significant percentage of the population. For example, a genetic polymorphism in the metabolism of the anticonvulsant drug S-mephenytoin has been attributed to defective CYP2C19 alleles. This genetic polymorphism displays large interracial differences with the poor metabolizer (PM) phenotype representing 2-5% of Caucasian and 13-23% of Oriental populations. Ibeanu et al. (J. Pharmacol. Exp. Ther.) 286(3): 1490-5 (1998). Several individuals showing poor metabolic capacity to coumarin and (+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolidin-4-one hydrochloride show very low levels of the CYP2A6 gene product. See Nunoya et al. (Pharmacogenetics) 8(3):239-49 (1998).

There are multiple additional examples of reported variation in genes known to be important in toxic responses, but clinical investigation has not yet been performed to determine their relative susceptibility to specific drugs. The human UDP-glucuronosyltransferase (UGT1A) locus is regulated in a tissue specific fashion in liver and extrahepatic tissues. Activity assays demonstrated 2- to 4-fold inter-individual differences in UGT activity and qualitative differences between individuals. The polymorphic regulation of UGT1A gene products in gastric tissue may be the biological basis that

determines inter-individual differences in extrahepatic microsomal drug metabolism. Strassburg et al. (Mol. Pharmacol.) 54(4):647-54 (1998).

Very importantly, it is likely that many mutations in single genes result in altered expression of many more genes, an amplification effect. A knock-out mutant has been created in mice that destroyed the function of a single gene, the au-beta 6 gene. The resulting animals showed altered basal expression of 101 genes in lung epithelial cells. Kaminski et al. (New York Academy of Sciences meeting, Toxicology for the Next Millenium, Airlie VA, USA) September 1999.

Single mutations in any one of hundreds of key toxicity genes can potentially cause differential basal levels of expression of many additional genes. It may be the altered expression of these genes that render the cell, or organism sensitive to toxic stress, not the initial mutation by itself.

Gene expression analysis has been used to predict who will respond beneficially to the therapeutic effects of treatments. The levels of Bax and Bcl-2 expression after radiotherapy have been used as prognostic markers in patients with human cervical carcinoma. Harima et al. (J Cancer Res Clin) Oncol 124(9): 503-10. (1998). In acute myeloid leukemia, coexpression of at least two proteins, including P-glycoprotein, the multi-drug resistance-related protein, bcl-2, mutant p53, and heat-shock protein 27, have been reported to be predictive of the response to chemotherapy. Kasimir-Bauer et al. (Exp Hematol) 26(12): 1111-7 (1998). The work by Kasmir-Bauer et al. shows that gene expression profiling can be used to predict who will benefit from the therapeutic effects of a drug; it does not address the question as to who will suffer enhanced toxicity of a drug.

All of the above examples show that altered levels of gene expression of a certain set of tox-response genes are associated with qualitatively or quantitatively distinct responses to the toxic effect of different drugs. Many of the examples show that DNA sequence polymorphisms would not be sufficient to predict hypersensitive individuals. Finally, the disclosure and examples herein show that measurement of a multiple set of tox-

response genes will reveal patterns of gene expression that will identify hypersensitive individuals for specific types of toxicity.

Experimental Identification of Genes

Genes associated with hypersensitivity to an agent may be identified in a variety of ways experimentally. Generally the expression of genes that are differentially expressed in total across cell, organ and tissue types in humans, in particular in response to toxic insult is evaluated to determine genes associated with hypersensitivity in an individual. In one embodiment, a method of identifying genes associated with hypersensitivity to an agent is provided, that comprises comparing the gene expression profile of cells treated with an agent with the gene expression profile of untreated cells, and determining genes in the treated cells that have altered expression due to the treatment, thereby to identify one or more genes associated with hypersensitivity to the agent. The cells may comprise one or more different cell types, wherein each said cell type comprises a gene associated with hypersensitivity to the agent. Alternately, the cell types are derived from a single tissue or organ.

Exemplary cell types are those derived from a specific organ , cell or tissue, such as kidney, liver, lung, heart, breast, lymphocytes, neuronal cells, skin, or intestine, such as HepG2, Caco-2, MCF-7, Jurkat, Daudi, HL-60, MCL-5, SKBR-3, SKOV-3, PC-3, WISH and HeLa.

Another method of identifying genes having a pattern of differential gene expression indicative of hypersensitivity to an agent comprises comparing the gene expression profile of multiple cell types of an individual known to be hypersensitive to an agent with the gene expression profile of said cell types in an individual known not to be hypersensitive to the agent; and identifying genes from said multiple cell types having a pattern of differential gene expression, wherein the pattern of differential gene expression is associated with hypersensitivity to the agent.

An alternative to this method comprises, comparing the gene expression profile of multiple cell types of an individual known to be hypersensitive to an agent before treatment with the agent with the gene expression profile of multiple cell types of the hypersensitive individual after treatment with the agent, and identifying genes from the multiple cell types having a pattern of differential gene expression, wherein the pattern of differential gene expression is associated with hypersensitivity to the agent.

When normal or hypersensitive animals, humans or cells are exposed to a selected agent, gene expression changes can be analyzed in genes such as those listed in Table 3, 4, 5, 6, 8, 10 and 11. Different types of toxic insult lead to different patterns of gene expression changes in normal, as well as in hypersensitive individuals. Since substantially all compounds elicit toxicity at a high enough dose, the mechanisms of drug toxicity in normal individuals has been well examined. Genes that cells induce to combat the toxic effect of various compounds are important for anti-toxicity for each compound. Patterns of gene expression of these genes in individuals who show hypersensitivity to a given compound that differ from the pattern of differential expression of normal individuals, with or without treatment can be identified. Using these methods, sets of genes that have characteristic expression in hypersensitive individuals that differs from normal individuals may be identified.

Subsets of genes and expression profiles thereof that can be used to identify hypersensitive individuals are identified as follows. A technique such as amplified fragment length polymorphism (AFLP) or serial analysis of gene expression (SAGE), which are known in the art, is used to compare gene expression profiles from treated and untreated human cells. The agent is administered at a toxic dose. This procedure identifies all candidate genes within the cells that respond to the toxic stimuli posed by a particular agent. The method further comprises using a technique, such as AFLP or SAGE, which are known in the art, to compare the gene expression profiles from treated and untreated normal cells. This step would identifies all genes within an individual that respond to that

agent. It also permits investigators to understand the normal expression range of individuals who are not hypersensitive. A technique, such as AFLP or SAGE, is used to compare gene expression profiles for samples from treated and untreated hypersensitive individuals or cell cultures derived therefrom. This step identifies all genes within hypersensitive individuals that respond to the treatment by that agent. It also allows investigators to understand the expression range of hypersensitive individuals. This permits identification of the genes that were differentially expressed in all of the above experiments, thus eliminating genes associated with therapeutically beneficial effects and individual variation in expression of genes unrelated to the compound. The expression of these genes can then be measured in a larger population of normal and hypersensitive individuals using, for example gene arrays, RT-PCR or other techniques known in the art to confirm the correlation between those genes identified in the above procedures and hypersensitivity observed in particular individuals.

Gene expression responses to toxic stimuli can be analyzed using a database of information. The first method is to determine which genes are induced and what is their function. For example, if all genes induced by a compound are regulated by DNA damage, the interpretation is that the compound causes DNA damage. This interpretation requires a database about the function and regulation of all genes in the database. Another method of interpretation is to determine whether the gene expression pattern induced by a second compound is similar to that induced by a compound, the toxicity of which is well-characterized. This approach to interpretation requires an extensive database of gene expression profiles generated from well-characterized compounds. Table 7 shows a partial list of well-characterized compounds for which gene expression data has been generated.

The methods of gene expression analysis discussed herein can be performed using a computer system with computer code suitable for accessing and comparing the gene expression profile determined according to the methods of this invention. Suitable software will also rank the results of these analyses. Computer code suitable for these

purposes can be programmed by a person skilled in the art. Exemplary software and a gene expression profile database related to toxicology are commercially available from Phase-1 Molecular Toxicology, (Santa Fe, NM), for example, Chem Profiler™ and Matrix Express™.

Examples of Genes Associated with Hypersensitivity

Several drugs have been shown to elicit allergic reactions in a subset of the population. The more extreme form of these allergic reactions can be quite severe and involve extensive damage of significant portions of the skin covering the body. Many patients die from dehydration and infection. The extreme form of these allergic reactions have the names Steven Johnson Syndrome and TEN (Toxic Epidermal Necrosis). Drugs known to elicit Steven Johnson Syndrome and TEN and less severe forms of skin allergy include navirapine, dapsone, acebutolol, trimethoprim, sulfasalozine, sulfacetamide, sulfadiazine, sulfamethoxizole, sulfasoxazole, sulfamethizole cotrimoxazole, amoxacillin, phenytoin, sulfonamide and penicillin.

Gene expression data suggests that the expression levels of a relatively small number of genes can identify who will develop allergic reactions to these drugs. The genes whose expression in CD8 T Cells and keratinocytes is likely to identify hypersensitive individuals include: inducible NOS, Ki-67, Transglutaminase-1, IL-1, FASL, TNF-alpha, CD11b/CD18, p75-R-TNF (TNF Receptor), IL-6 receptor, G-CSF receptor, HSP-70, INF-gamma, ICAM-1, VCAM-1, ECAM-1, and TGF-beta.

While not being limited to any theory, it is believed that there is a similar molecular mechanism for both Steven Johnson Syndrome and TEN. The invention provides a method to determine who will develop these syndromes prior to taking these drugs.

All publications, patents, and patent applications referred to herein are incorporated herein by reference.

The following examples are intended to illustrate but not to limit the invention.

EXAMPLES

Example 1: Identification of Genes Associated with Hypersensitivity and Screening of Subjects Prior to Drug Administration

Initially, one or more compounds predetermined to cause blood toxicity, such as agranulocytosis, in at least 4% of the patient population, are chosen, e.g. Haldol™ (haloperidol). The differential gene expression profile associated with Haldol™ (haloperidol) is determined in neutrophils from both normal and hypersensitive subjects when exposed to high concentrations of Haldol™ (haloperidol). The gene expression profile from untreated and treated cells is compared using for example, AFLP, a microarray of the genes listed in Tables 3 and 4, or SAGE, to identify genes that vary as a function of toxicity and vary as a function of hypersensitivity to the Haldol™ (haloperidol). Next, gene expression from clinical samples from a patient population exposed to Haldol™ (haloperidol) or a placebo is measured. The clinical samples are provided by the manufacturer of Haldol™ (Hoechst Marion Roussel). Genes are identified that co-varied with the hypersensitivity status. Additional clinical samples are blinded and provided by the manufacturer which includes samples from normal and hypersensitive subjects. Using the present invention, prediction of the hypersensitivity status is based upon gene expression profiles. The level of accuracy of the prediction or correct identification is determined by unblinding the compounds.

In the final stage, gene expression analysis of the key set of genes would be performed on a prospective basis with new patients just beginning treatment with Haldol. The level of accuracy of the prediction or correct identification of hypersensitivity is determined by monitoring patients over time to see if those predicted to develop

agranulocytosis indeed did so. This empirical approach is then be extended to other drugs and other drug manufacturers.

Example 2: cDNA Probe Production

A fluorescent dye labeled cDNA probe complementary to the mRNA component of cellular RNA harvested from cells exposed to toxicologic challenge is produced by this protocol, which is designed to produce sufficient Cy3 labeled probe from one experimental sample, and Cy5 labeled probe from one control sample, to develop one microarray slide. The procedure is scalable to easily accommodate, for example, 16 samples. This will produce sufficient probe mixtures for at least 8 microarray slides. General procedures as described, for example, in Gerard et al. (Focus®) 14:91 (1992); Kotewitcz et al. (Gene) 35: 249 (1985); and Gerard et al. (DNA) 5: 271 (1986) are utilized.

cDNA probes may be used in an assay for detecting expression of genes associated with hypersensitivity to an agent. In one embodiment, microarray slides are provided that contain ssDNA sequences, or targets, from a number of toxicologically relevant genes. The microarray slides, for example, may be 3"x 1" glass microscope slides comprising an array of micron-scale spots of ssDNA sequences on the upper face. The DNA may be bound to the slide using covalent linkage chemistries known in the art.

Total RNA from cells contains mRNA species that are homologous to these sequences. "Total RNA (high quality)" refers to substantially total cellular RNA. As RNA is very labile, special care must be taken to insure that it is of sufficient integrity at the time of use as template in the production of probe. The level of these mRNA species is proportional to the degree of induction of the gene by the agent under study. This protocol describes the production of fluorescent labeled cDNA probe from the total RNA of cells which have either been exposed to the agent under study, or are serving as a non-treated control. These probes are then pooled and hybridized to the microarray slide. The experimental and control probes are distinguishable because the Cy3 and Cy5 labels

fluoresce at different wavelengths. The degree to which each probe binds to a specific gene sequence on the slide reveals the level of induction of that gene in the cells exposed to the agent under study.

The following materials are used:

Material	Amount	Exemplary Source
DEPC treated water	10ml	Ambion®
Alk Water (pH 7.5 with NaOH)	1ml	
Total RNA (of high quality) or	10µg/sample	
Messenger RNA (of high quality)	2µg/sample	
First strand buffer	4µl/sample	
0.1 M DTT	2µl/sample	Sigma®
1:8 dilution Cy3 dCTP	1µl/exp. Sample	Amersham®
(3-amino-propargyl-2'-deoxycytidine 5'-triphosphate) (i.e., 0.125mM Cy3 dCTP)		
1:10 dilution Cy5 dCTP	1µl/control sample	Amersham®
(3-amino-propargyl-2'-deoxycytidine 5'-tiphosphate) (i.e., 0.1mM Cy5 dCTP)		
SuperscriptII (RT)	1µl/sample	Life Technologies, Inc.
ANTI-RNase	1µl/sample	Ambion®
7.5 M ammonium acetate	34µl/sample	Sigma®
70% EtOH	1ml/sample	J. T. Baker®
95% EtOH	220µl/sample	J. T. Baker®
Nucleotide Mix "3"	1µl/exp. sample 0.5 mM dATP/dGTP/dTTP 0.125 mM dCTP	
Nucleotide Mix "5"	1µl/control sample 0.5 mM dATP/dGTP/dTTP 0.15 mM dCTP	
Stock anchored oligo dT:	4µl/sample 0.25µg/µl of each oligo dT (in Water @ -20°C)	
RNase Zap	(1) bottle The RNA Co. ™	
Wet ice	(1) bucket	
Qiagen Qiaquick PCR purification kit	(1) ea Qiagen®	
PE/ETOH	(1) bottle (100 ml PE buffer + 400 ml >96% EtOH)	
EB Buffer	10 ml (10 mM Tris-HCl pH 8.5)	

General Protocol

Steps are performed at room temperature unless otherwise specified. Work areas are cleaned and swabbed with RNase Zap. Gloves are worn at all times. RNase (RNA specific endo-and exo-nucleases) is a ubiquitous and very stable enzyme. Standard cleaning and/or autoclaving will not remove or inactivate it. Therefore all materials contacting the samples must be known RNase-free. All water, including for buffers, must be DEPC-treated. DEPC treatment consists of an autoclaved solution of 0.1% Diethyl pyrocarbanate in de-ionized water.

Preparation of RNA template in water is implemented by adjusting mRNA to a concentration of 2μg/7μl or total RNA to a concentration of 10μg/7μl for each sample in a standard microfuge tube. If concentration adjustment requires dehydration in the Speedvac™, 1 μl Anti-RNase is added prior to dehydration. The reaction solution is prepared by adding 4 μl of stock anchored oligo dT per tube, heating at 70°C for 10 minutes in a heat block, spinning 5 seconds in microfuge, and placing on ice for 2 minutes. The following is then added to each tube:

4 μl 5x First Strand Buffer for SuperscriptII

2 μl 0.1 M DTT

and either (for treated samples):

1 μl Nucleotide Mix "3"

1 μl of 1:8 dilution of Cy3

or (for control samples):

1 μl Nucleotide Mix "5"

1 μl of 1:10 dilution of Cy5.

The tube then is incubated at room temperature for 10 min. The dCTP is added to limit the concentration of Cy dCTPs incorporated. Due to the size of the Cy dCTP, the polymerase will fall off the template if more than two are incorporated in a row.

To perform the reaction, 1 µl SuperScriptII is added to each tube, and the contents mixed gently. The tube then is incubated for 1.5-2 hr. at 45°C in a heat block, keeping the reaction protected from light. The fluorescent dyes Cy3 and Cy5 are sensitive to light. Excessive exposure during processing will reduce the intensity of emission upon final scanning.

To collect the labeled cDNA probe, ethanol precipitation is implemented by adding to each tube 46µl of water, 34µl of 7.5M ammonium acetate and 220µl of 95% EtOH, and then incubating at –80°C for 15-20 min. If desired, procedure may be interrupted at this point. The sample may be stored at –80°C for up to 7 days.

The tubes are loaded in centrifuge with orientation of lid noted, centrifuged for 15 min at 20800 x *g*, and the supernatant discarded, to obtain a visible pellet (pink for Cy3, blue for Cy5). The pellet is washed by adding 750µl 70% EtOH per tube and vortexing briefly, centrifuging at 20800 x *g* for 10 min, decanting and discarding the supernatant, centrifuging the pellet and optionally gently removing remaining EtOH with a pipette, while being careful not to loosen the pellets. The pellet is allowed to dry for 10 min. at room temp, but not over drying by using a vacuum, and resuspended in 40µl water. cDNA/mRNA hybrid is denatured by incubating at 95°C for 5 min. in a heat block. The tube then is spun 5 seconds in microfuge.

The labeled cDNA probe is purified in an adaptation of the procedure described on page 18 of the QIAquick Spin Handbook, (1997) Qiagen®. To bind the cDNA probe to a column, 200 µl of Buffer PB is added to each 40µl probe solution, the QIAquick spin columns are placed in 2 ml collection tubes, and the samples are applied to the QIAquick columns and centrifuged at 10,000 x g for 2 min. The flow-through is discarded and QIAquick columns replaced into the same tubes.

To wash bound cDNA probe, 750µl Buffer PE/ETOH is added to each column, and the column incubated for 1 min. at room temp. The column is centrifuged at 10,000 x g for 2 min., and the supernatant discarded. The wash is repeated. QIAquick columns are

placed back in the same tubes, and centrifuged for an additional 1 min at maximum speed with tube lids open. Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

QIAquick columns are placed in clean 1.5ml microfuge tubes. To elute the cDNA probe, 40µl (+/- 10µl) Alk. Water is added to the center of each column. The tubes are incubated for 1 min, centrifuge at 6000 x *g* for 1 min., and the elution steps repeated once into same tube. The elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound cDNA.

To quantify the cDNA probe, each sample is put in ~80µl of EB buffer, and transferred to one well of a 384 well plate. Scanning, including the measurement and recording of the type and degree of fluorescence from each spot on a processed microarray slide, is accomplished in a confocal laser scanning fluorimeter. The fluorimeter is set to the appropriate excitation/emission frequencies and records the level of emission for the sample. The exposure time and intensity is controlled, because exposure of the label to strong light incrementally reduces its fluorescent activity. Values from this procedure are the result of many variable factors. Therefore it is preferable to compare to an archive of values produced from the same procedure and equipment.

To prepare the final probe mixture, the Cy3 labeled experimental probe is combined with the Cy5 labeled control probe. If a control requires multiple reactions, they are combined prior to aliquoting equal amounts to the experimental samples. The combined probes are concentrated to ~1µl in a Speedvac at a temperature not exceeding 45°C. If the probe is not used immediately, 10 µl water is added and it is stored at 4°C.

Example 3: Determination Of Gene Expression Changes Associated With Toxicity

To determine genes useful for identifying patterns of genes associated with toxicity, animals were exposed to concentrations of selected compounds that elicit peroxisome proliferation, a type of liver toxicity. Treatments were with WY 14,643, gemfibrozil and

clofibrate in Sprague Dawley rats. Each compound was administered in 1% carboxymethycellulose/0.2% Tween 80 by oral gavage daily for 14 days. Administered doses were to three animals per dose per time point as follows; WY14,643, 40 mg/kg/day; gemfibrozil, 24 mg/kg/day and 100 mg/kg/day, and clofibrate 40 mg/kg/day and 250 mg/kg/day.

Gene induction was measured using microarrays consisting of 250 toxicologically relevant rat genes using the hybridization protocol described above. As illustrated in Figure 4, several genes were induced by the treatment, example given is for WY14,643. This figure shows a gene expression profile showing the relative induction levels compared to untreated controls. As shown in Figure 4, the genes referred to in Figure 4 as A (Cytochrome p450 4A *CYP4A*, B (Enoyl Co-A Hydratase), C (3-ketoacyl CoA thiolase 2), D (Acyl CoA Oxidase), and E (Ketoacyl CoA thiolase type 1), had enhanced expression in comparison to the control after treatment with the compound. These genes were found to be induced by a variety of other peroxisome proliferating agents including gemfibrozil, clofibrate, fenofibrate and DEHP. This set of genes was thus empirically shown to be induced by a variety of compounds that exhibit a specific type of hepatotoxicity, peroxisome proliferation. By way of example, individuals who display hypersensitivity to these types of compounds should show altered expression of this set of genes.

Example 4: Probe For Hepatocyte Growth Factor

New genes associated with and predictive of toxicity were identified. Different types of damage to the liver cause the formation of dead and dying hepatocytes, which the liver replaces to maintain its function. Induction of the hepatocyte growth factor receptor gene by toxic stimuli in both rats and humans was examined. When several nitrosoureas including streptozotocin, carmustine and MNU were used to determine gene expression profiles, all of these compounds induced several genes in common. These compounds are all known to form covalent adducts to the DNA in liver and liver cells. All compounds, for

example induce both the hepatocyte growth factor receptor gene and the glutathione transferase gene. Exemplary data is provided in Figure 1 which shows the gene expression profile in the liver of male Sprague-Dawley rats when treated with the hepatotoxicant streptozotocin.

The probe for the hepatocyte growth factor receptor gene was created by cloning at least a 250 base-pair section from the 3' coding region of the gene starting with total genomic DNA. The fragment was derived by PCR from genomic DNA using two primer with appropriate linkers for insertion into a plasmid vector. A single stranded probe complementary to the cDNA sequence was attached to a glass slide array using a polyamine attachment.

In more detail, an example of creation of a specific probe for the hepatocyte growth factor receptor is as follows. The first step in the process is obtaining the sequence for the gene. The search for gene sequence, either by gene name or accession number, is performed using the NIH National Center for Biotechnology Information website using Genbank (http://www2.ncbi.nlm.nih.gov/genbank/query_form.html). The accession number for the rat hepatocyte growth factor receptor gene is X96786. When the sequence of interest is located, the sequence information is copied to a Microsoft Word file. Intron sequences are then removed, if present, as well as numbers and white spaces. The resulting condensed sequence is then submitted to a PCR primer design software program, such as Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). Primers are selected that optimally have a T_m in the range of 60°-63°C. The optimal length of the gene fragment is 500 bp. Shorter fragments are chosen if the starting sequence is shorter than 500 bp. Once the primers are designed, the sequence that is flanked by the primers is submitted to a BLAST search. BLAST (Altschul et al (Nucleic Acid Res) 25: 3389-3402. (1997)) is a sequence analysis software program supported by the NIH. The BLAST search software searches for other DNA sequences that are homologous to the target sequence and ranks these sequences according to the amount of homology. This ensures that the chosen gene

fragment sequence will not cross-hybridize with a gene sequence other than the desired sequence. PCR primers are ordered and an attempt is made to isolate the gene fragment from a cDNA library that is created by reverse transcription of RNA from either a cell line(H4IIE) or rat tissue. Upon identification of a PCR band of the correct size, the PCR product is cloned into a vector (TA cloning vector, Invitrogen Corp., Carlsbad, CA). Following cloning, a bacterial mini-prep is performed to amplify and isolate the plasmid containing the gene fragment of interest. The region of the plasmid containing the gene fragment is then sequenced. If this sequence matches the original target sequence, the target sequence of this clone is amplified by PCR, purified (Wizard system, Promega Corp., Madison, WI), quantified, and used for spotting.

The probe refers to a population of cDNAs bearing fluorescently active ligands which are produced from the mRNA of the cells under examination, while "probe mixture" refers to a mixture of two or more populations of cDNA. The cDNAs may also be labeled with a variety of ligands, such as fluorescently active ligands, radioisotope ligands or biotinylated ligands.

Example 5: Glutathione Transferase Positive Foci

Enhanced gene expression and co-induction of genes associated with the formation glutathione transferase positive foci was identified.

Certain types of toxic liver damage produce glutathione transferase positive foci Lemmer et al. (Carcinogenesis) 20:817-824 (1999) which are cells that are in the late stages of dying. In response, neighboring hepatocytes must replicate in order to replace the dying cells and induce expression of hepatocyte growth factor so that they are 'primed' for growth hormone signals.

Co-induction of the glutathione transferase and hepatocyte growth factor receptor genes was determined by hybridization to microarrays containing at least 300 human toxicologically relevant genes using the hybridization protocols described above.

Figure 2 is a graph showing the results, which indicated a very strong correlation between the induction of the glutathione transferase and hepatocyte growth factor receptor genes. Co-induction thus shows correlation to focal cell death occurring in the liver.

Example 6: Heart Muscle Tissue Gene Expression Profile

Animals were exposed to doxorubicin, a cardiotoxin. Male Sprague-Dawley rats were treated with 1 mg/kg doxorubicin in 5% saline for 6 ho 24 hours, and 7 days and 6 weeks with one dose per day and a 6 week recovery period. A gene expression profile of heart muscle tissue cells was then obtained. RNA was isolated and the gene expression profile was analyzed as described below. Gene expression of all genes listed in Table 8 was determined. The results of the gene expression of the first 66 genes is shown in Figure 3.

As can be seen from Figure 3 and Table 8, several genes, including activating transcription factor 4, activin receptor type II, ataxia telangeictasia, c-jun, carnatine palmitoyl-CoA transferase, DNA Dependent helicase, Epozide hydrolase, farnesol receptor, Gadd 45, Interleukin 6, MDM-2, Ribonucleotide reductase subunit M1 and at least 10 others were differentially expressed at significant levels. Many of these genes, including Carnatine Palmitoyl transferase, Epoxide hydrolase, Farnesol receptor, Lipoprotein lipase precursor, and MDM-2 have never been reported or previously known to be induced by cardiotoxicity.

Thus a profile of gene expression characteristic of the cardiotoxin, doxorubicin was obtained. Genes thus identified as having altered expression in the presence of cardiotoxin are significant, because individuals with diminished or altered expression of the induced genes may potentially be hypersensitive to the toxicity of doxorubicin. Such

hypersensitivity could manifest itself at the molecular level as altered induction of these genes as well as a shift in the dose-response curve such that the same genes would be induced at lower concentrations.

Example 7 Determining genes associated with hypersensitive reaction to penicillin

Three different methods, differential display, microarray technology, and Taqman® assay were used to determine genes associated with hypersensitive reaction to penicillin. Seven self-described penicillin-sensitive individuals and six individuals self-described to have normal reaction to penicillin were tested by differential display. Six self-described penicillin-sensitive individuals and six individuals self-described to have normal reaction to penicillin were tested by microarray technology.

1. Lymphocyte culture

Six individuals self-described as penicillin sensitive and seven individuals self-described as having normal reaction to penicillin were used to determine potential hypersensitive reactions to penicillin in humans. Peripheral blood leukocytes (PBL) were isolated from a population of individuals, cultured with PHA at a standard concentration for culturing lymphocytes for 24 hours, washed, cultured for another 24 hours without PHA, and divided into two groups. One group was exposed to penicillin *in vitro* for 24 hours and the other group was not exposed to penicillin as a control group. At a non-toxic dose of 1250 μg/ml, penicillin G is known to elicit an immune response in peripheral blood of individuals with proven penicillin G allergy.

2. Isolation of RNA from cultured lymphocytes

RNA from select individual from both groups (treated and untreated) of cultured lymphocytes was isolated as follows. Total RNA of high quality and high purity is isolated from cultured cells by using Qiagen QIAamp® RNA blood mini kit and 2-

mercaptoethanol. RNA degradation by RNases is not desirable when synthesizing fluorescent cDNA for hybridization with the penicillin array. Precautions are taken to minimize the risk of RNA degradation by RNases by wearing gloves, treating work areas and equipment with a RNase inhibitor, for example, RNase Zap (Ambion® Products, Austin, TX) and keeping samples on ice. This total RNA isolation technique is based on a Qiagen QIAamp®RNA blood mini kit and is used with some modification for human lymphocyte cells in a T-75 flask.

Cells are checked under the microscope to make sure that they are viable. Cells are dosed with penicillin on the third day in culture (48 hours after introduction of the cells into culture).

Cells are scraped from the flask and poured into a 50 ml conical tube. The flask is then rinsed with 10 ml of room temperature PBS. The PBS wash is removed with a pipette. The tube is then spun for 10 minutes at 1,000 rpm and the supernatant pipeted off. The remaining pellet is resuspended in 600µl of freshly prepared RLT buffer (RLT buffer requires the addition of 10µl of beta mercaptoethanol for each 1.0 ml RLT) by vortexing. The resuspended pellet is pipeted into a QIAshredder® column and centrifuged for 2 minutes at 14,000 rpm in a Eppendorf® 5417C centrifuge. The QIAshredder® column is discarded and 600 µl of 70% ethanol added to the lysate. The lysate is then pipeted into a QIAamp® spin column sitting in a 2 ml collection tube and centrifuged for 15 seconds at 14,000 rpm. Any remaining lysate is placed on the same column and the centrifugation is repeated. The QIAamp® spin column with the RNA bound to the column is transferred to a new 2 ml collection tube. 700µl of Qiagen® RW1 buffer is added to wash the column and centrifuged for 15 seconds at 14,000 rpm. The QIAamp® spin column is transferred to a new 2 ml collection tube. 500µl of Qiagen® RPE buffer is added to the column and centrifuged for 15 seconds at 14,000 rpm. The QIAamp® spin column is transferred to a new 2 ml collection tube. 500µl of Qiagen® RPE buffer is added to the column and centrifuged for 3 minutes at 14,000 rpm. The QIAamp® spin column is transferred to a

new 2 ml collection tube and centrifuged for 1 minute at 14,000 rpm. The QIAamp® column is transferred to 1.5 ml microcentrifuge tube and 50 µl of RNase-free water is added to the column and centrifuged for 1 minute at 14,000 rpm. An additional 50 µl of RNase-free water is added to the column and centrifuged for another 1 minute at 14,000 rpm.

To measure the yield, the O.D. reading is taken at 260nm on a Beckman DU®350 UV vis spectrophotometer. 1.0 µl RNA is added to 49 µl of sterile nanopure water and the O.D. reading is taken and calculated as follows:

(Absorbance) x (dilution factor) x (40)/1000 = amount of RNA in µg/µl

Example: absorbance = 0.45

Dilution factor = 50

$\frac{(0.45) \times 50 \times 40}{1000}$ = RNA concentration in µg/µl

The sample is stored in -80°C freezer.

3. MessageClean® of Total RNA

It is important that total RNA that is used to make mRNA differential display is absolutely free of DNA contamination. Regardless of the method used for RNA isolation, a cleaning step is important to ensure the removal of DNA contamination, especially if the differential display banding pattern on the denaturing polyacrylamide gel is independent of the reverse transcription step. MessageClean® from GenHunter (Nashville, TN) was used to clean total RNA. Components for twenty RNA sample cleanings included the following materials: 140 µl 10x Reaction Buffer, 20 µl GH-DNase I (RNase free, 10 units/µl), 140 µl 3M NaOAc, and 1 mL DEPC-treated H_20. For DNase I digestion, the following materials were added in order: 50 µl total RNA (10-50 µg), 5.7 µl 10x Reaction Buffer, 1 µl DNase I (10 units/µl) for a total volume of 56.7 µl. The materials were mixed well and incubated at 37 degrees for 30 minutes. A 3:1 phenol/chloroform mixture is used to ensure removal of

protein contamination and DNase I from the RNA. About 40 µl of phenol/chloroform is added to the mixture, vortexed for 30 seconds, and allowed to sit on ice for about 10 minutes. Then the mixture was spun in an Eppendorf centrifuge at 4 degrees for 5 minutes at maximum speed and the upper phase of the mixture is collected. Ethanol precipations was performed as follows. About 5 µl of 3M NaOAc and 200 µl of 100% ethanol was added to the upper phase that was collected. This was placed at -80 degrees for more than 1 hour and then spun for 10 minutes at 4 degrees. The supernatant was removed, the RNA pellet was washed with 0.5 mL of 70% ethanol (in DEPC-treated water), and spun for 5 minutes to remove the ethanol. The tube containing the materials were spun again and the residual liquid was removed. The RNA was re-dissolved in 10-20 µl DEPC-treated water. The RNA was quantitated by reading on a spectrophotometer at OD_{260}. RNA that is diluted for any purpose, such as quantitation, should not be re-used after freezing and thawing. The integrity of the RNA can be checked by running a few micrograms on a 7% formaldehyde agarose gel and looking for the clear appearance of 28S and 18S rRNA bands.

4. Reverse transcription

In a tube, the following ingredients are added: 9.4 µl dH_20, 4.0 µl 5x RT buffer, 1.6 µl dNTP (250 µM), 2.0 µl of 0.1 µg/µl freshly diluted total RNA that is DNase-free, 2.0 µl H-T_{11}M (2 µM) for a total volume of 19 µl. The ingredients are mixed well and incubated at 65°C for 5 minutes, 37°C for 60 minutes, 75°C for 5 minutes, and held at 4°C. After the tubes had been at 37°C for 10 minutes, and 1 µl of SuperScript II reverse transcriptase (Life Technologies Inc.) is added to each reaction, and quickly mixed by finger tapping the tubes before the incubation continued. At the end of the reverse transcription, the tubes are spun briefly to collect condensation. The tubes are set on ice for PCR or stored at -20°C for later use.

5. PCR to amplify gel band

The following is an exemplary protocol for PCR. The following ingredients are used: 10 µl dH_20, 2 µl 10x PCR buffer, 1.6 µl DNTP (25 µm), 2 µl of 2 µm H-AP primer, 2 µl of 2 µm H-T_{11}M, 2 µl RT-mix described above (must contain the same H-T_{11}M used for PCR), 0.2 µl α-^{33}p DATP (2000 ci/mmole), 0.2 µl TAQ DNA polymerase from PE Biosystems for a total volume of 20 µl. The tube containing all these ingredients are mixed well by pipeting up and down and placed in a thermocycler at 95°c for 5 minutes and then amplified for 40 cycles under the conditions of 94°c for 30 seconds, 40°c for 2 minutes, 72°c for 30 seconds and finally held at 4°c until the samples are removed from the thermocycler.

6. Gel electrophoresis

RNA was analyzed by gel electrophoresis to identify possible candidate genes. A 6% denaturing polyacrylamide gel in TBE is prepared and allowed to polymerize for at least 2 hours before using. Then the gel is run for about 30 minutes before any samples are loaded. It is important for all the sample wells in the gel to be flushed and cleared of all urea prior to loading any samples in the wells. About 3.5 µl of each sample is mixed with 2 µl of loading dye and incubated at 80°C for 2 minutes immediately before loading onto the 6% gel. In this example, the loading dye is xylene and after the gel is loaded with the samples obtained from the rounds of PCR, the gel is run at 60 watts of constant power until the xylene dye is about 6 inches from the bottom of the gel. Once the power is turned off, the gel is blotted onto a large sheet of exposed autoradiograph film. The gel is covered with plastic wrap and under dark conditions, the gel is placed in a large autoradiograph cassette with a new sheet of unexposed film, marked for orientation, and the film is allowed to be exposed to the gel at -80°C. The exposure period can be anywhere from overnight to 72 hours. Once the film has been developed, bands of interest, which show differential expression between penicillin sensitive and normal individuals, are identified by alignment

with the developed film and subsequently isolated by cutting the band of interest out of the polyacrylamide gel with a clean scalpel blade. The isolated band is placed in 100 µl of water and boiled at 95% for 5 minutes.

7. Cloning re-amplified PCR products for differential display

The following procedure was used to clone re-amplified PCR products from differential display. Material which may be used include the PCR-TRAP® Cloning System (GenHunter®). For a 20ul Ligation reaction, add in order: 10ul dH2O; 2ul 10X ligase buffer; 2ul Insert-ready PCR-TRAP® Vector; 5ul PCR product; 1ul T4 DNA ligase. The reaction is mixed well by finger tipping and is briefly spun. Then the reaction is ligated overnight at 16°C. The reaction can then be used directly for transformation or stored at -20°C. For transformation, the GH-competent cells are thawed in ice water slush for 15 minutes. While the cells are melting, the appropriate number of 1.5ml microfuge tubes are labeled and set on ice. The cells are quickly mixed by finger tipping and are divided into 100ul aliquots into each 1.5ml microfuge tube. The remaining competent cells are immediately re-frozen for future use. The ligation tubes are spun briefly to collect condensation. About 10ul of each ligation mix is added to an above tube containing the competent cells and mixed well by finger tipping and incubated on ice for 2 minutes. About 0.4ml of LB medium is added and the cells are incubated at 37°C for 1 hour. It is important that no Tetracycline be in the LB during this step because the bacteria with recombinant plasmids need time to express the Tetracycline resistance gene. It is recommended that the LB-Tet plates are warmed at 37°C for 1 hour before plating. After vortexing briefly, about 200ul of cells are plated on an LB-Tet plate (containing 10ug/ml of tetracycline). For the lacZ control insert, about 200ul of cells are added to the plate. Then 30ul of X-gal is added to the middle of the cells and the cells are immediately spread onto the LB-Tet plate. Unplated cells can be stored at 4°C if replating is needed within 1 week. Once the plate surface is dry, the plate is incubated upside-down overnight at 37°C. The

Tet colonies are scored and the plate is save upside-down at 4°C. Three individual Tet resistant colonies are picked for each clone with a 10ul pipette tip, placed in labeled sterile culture tube containing 3ml of LB broth and grown overnight at 37°C.

8. Screening colonies for inserts

Plasmid DNA was isolated using the Qiagen Qiaprep Miniprep kit. PCR was used to check for inserts in the plasmids. For each colony the following PCR reaction mixture was set up:

dH2O	10µl
10xPCR buffer	2µl
dNTPs (250µM)	1.6µl
Left primer	2µl
Right primer	2µl
Plasmid DNA	2µl
Taq DNA Polymerase	0.2µl

The PCR parameters were 94°C for 30 sec, 52°C for 40 sec, 72°C for 1 min for 30 cycles followed by 5 min extension at 72°C and a final incubation at 4°C. All 20µl of the PCR product was analyzed on a 1.5% agarose gel with ethidium bromide staining.

Once the positive colonies were identified, they were sequenced by standard methods well-known to a skilled artisan. The sequences were compared to known sequences to determine if the sequence was already known.

As indicated at the beginning of Example 7, either differential display or microarray techniques were used to further determine genes related to penicillin hypersensitivity.

9. Genes Identified

By gel electrophoresis, about 260 candidate genes were identified and about 220 were cloned and sequenced to identify genes that predict hypersensitivity to penicillin. A summary of the genes associated with penicillin hypersensitivity is summarized in Table 10. Several new genes were identified that did not match any sequence listing in GenBank. Novel sequences which did not match any BLAST searches or GenBank searches are indicated in Table 10 under the "Identification " column as "no significant match to anything". Thus, provided herein are nucleic acids comprising said novel sequences and fragments thereof as well as amino acid sequences encoded therefrom and fragments thereof. Also provided are nucleic acids that hybridize to said novel sequences under stringent conditions. Such stringent conditions include conditions of a hybridization reaction that allow nucleic acid duplexes to be distinguished based on their degree of mismatch. Means for adjusting the stringency of a hybridization reaction are well-known to those of skill in the art. See, for example, Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, Second Edition, Cold Spring Harbor Laboratory Press, 1989; Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons,1996 and periodic updates; and Hames *et al.*, NUCLEIC ACID HYBRIDIZATION: A PRACTICAL APPROACH, IRL Press, Ltd., 1985. In general, conditions that increase stringency (*i.e.*, select for the formation of more closely-matched duplexes) include higher temperature, lower ionic strength and absence of solvents; lower stringency is favored by lower temperature, higher ionic strength, and higher concentrations of solvents (for example, formamide or dimethyl sulfoxide).

The following are some of the genes identified using the methods disclosed herein (GenBank identification numbers in parenthesis): hypothetical protein (HSPC004), UBA3 (UBA3) mRNA, clone CTA-732E4 on chromosome 22q12.1, ribosomal protein S7 (RPS7), myosin-binding protein C, cardiac (MYBPC3), CGI-51 protein mRNA, latexin mRNA, NADH oxidoreductase subunit MWFE, jun B proto-oncogene (JUNB), KIAA0787 protein, fatty acid synthase, polymerase (RNA) II (DNA directed) polypeptide B (140 kD), UbA52

gene coding for ubiquitin-52 amino acid fusion protein, small nuclear ribonucleoprotein 70kD polypeptide (RNP antigen) (SNRP70), isocitrate dehydrogenase 3 (NAD+) gamma (IDH3G), clone 565E6 on chromosome 11q12-1q22.2, hypothetical protein FLJ20436 (FLJ20436), c-Cbl-interacting protein L7a (RPL7A), ribosomal protein L7a (RPL7A), ribosomal protein S21 (RPS21), sorting nexin 6 (SNX6), TNF-inducible protein CG12-1 (CG12-1), BRCA2 gene region chromosome 13q12-13, CGI-128 protein mRNA, Tu translation elongation factor, mitochondrial (TUFM), KIAA0787 protein, ribosomal protein L13 (RPL13), ribosomal protein L19 (RPL19), clone 245M18 on chromosome 6p21.32-22.3, clone TCBA00781, chromosome 19 cosmid R26529, tumor suppressing subtransferable candidate 1 (TSSC1), transferrin receptor (TFRC), ubiquitin-conjugating enzyme E2D 3 (UBE2D3), putative DNA-directed RNA polymerase III C11 subunit, myosin-binding protein C (cardiac) (MYBPC3), tapasin (NGS-17), CoREST protein (COREST) (KIAA0071 protein), dynamitin (dynactin complex 50 kD subunit) (DCTN-50), alpla-L-fucosidase, metallothionein-IG (MT1G), Familial Cylindromatosis cyld gene, cDNA FLJ10589 fis (clone NT2RP2004389), eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein) (EEF1D), chromosome 16 BAC clone CIT987SK-A-67A1, proteasome (prosome, macropain) subunit beta type 8 (large multifunctional protease 7) (PSMB8), and lectin galactoside-binding soluble 9 (galectin 9). An unexpected result that was found was that there were apparently no p450 genes or metabolism genes that were gene candidates for penicillin hypersensitivity.

10. Gene Correlations

Gene expression profiles comprised of 180 genes on the penicillin array were compared for similarity between six penicillin-normal individuals and six self-identified penicillin-sensitive individuals. Three of the penicillin-sensitive profiles were repeat samples taken at different times. As shown in Figure 6, Samples 6005, 6015, and 6042 are from one individual, and samples 6041 and 6043 are from another individual. Using all genes for

comparison, sensitive individuals tend to resemble one another while non-sensitive individuals have little discernable pattern. The one exception is non-sensitive individual 6002, whose profile has some resemblance to the sensitive individuals.

In an exploratory analysis, independent-samples t-tests were performed to suggest which genes were differentially expressed between penicillin-sensitive and penicillin-insensitive individuals. Twenty genes in which the p-value of the t-test showed a statistically significant difference between the two classes at a level of 0.005 or less were identified as indicated in Table 11. Using the 20 genes identified as "discriminator" genes, the correlation between normal individuals and the discriminator genes were calculated as well as the correlation between sensitive individuals and the discriminator genes. The discriminator correlations are shown in Figure 7. Using the 20 discriminator genes and relevance network grouping, a similar correlation resulted. At a 0.9 correlation level, the only group that reveals itself is among the sensitive individuals. At a lower similarity level of 0.8, non-sensitive individual #2, who appeared to be borderline hypersensitive, joins the group of sensitive individuals. Methods of analyzing expression data statistically which are known in the art may be used, such as those described in "Family-Wise Error Rate", Glass, G. and Hopkins, K., Statistical Methods in Education and Psychology (1984), Prentice-Hall; and "Relevance Networks", Butte, A. J. and Kohane, I. S. (2000), Mutual Information Relevance Networks: Functional Genomic Clustering Using Pairwise Entropy Measurements. PSB00, 5:415-426.

Figure 8 shows that the 20 discriminator genes were analyzed for co-regulation, revealing several co-varying groups, as shown in both the similarity matrix and the relevance network grouping.

10. Preparation of penicillin arrays

In addition to differential display, microarray techniques were utilized to determine genes related to penicillin hypersensitivity. The following are methods that were used to

prepare microarray for testing for penicillin hypersensitivity. Of 260 potential gel band, 220 were cloned and sequenced. About 180 genes were put on a penicillin array, made as described below, and 20 discriminator genes (Table 11) were selected related to penicillin hypersensitivity.

Large Scale PCR (in 96-well plates)

For 1000 PCR reactions, 4X Master Mix can be made with the following materials:

10X PCR buffer	10 ml	
dATP	200ul (100mM)	
dGTP	200ul (100mM)	
dCTP	200ul (100mM)	
dTTP	200ul (100mM)	
Amine-linked vector primer (= "3X")	900ul (Forward or Reverse)	1ug/ul
Taq Polymerase	1 ml	5U/ul
H_2O	12.3 ml	
	25 ml total	

About 2.5ml aliquots are put into 15ml conical tubes and store at –20°C. One tube is enough for 1 96-well plate of PCR. Alternatively, about 12.5ml aliquots can be used in 50ml conical tubes, which is enough for 5 plates of PCR. dNTPs was obtained from Pharmacia Ultrapure dNTP set, cat#27-2035-02 (set contains all 4, 1ml each) and Taq Polymerase was obtained from Perkin Elmer N808-0155 (comes with 10X buffer). Template and gene-specific primer mix was made for 2 rows, or 16 wells by utilizing the following materials: 400ul H_2O, 2.5ul plasmid, 15ul of 1ug/ul gene specific primer.

To perform PCR, the following steps were performed:

1. Take one tube of PCR master mix and add 2 volumes of water (i.e., add 5ml water to 2.5ml MM).
2. Using a multichannel pipette, distribute 75ul MM to each well of a 96-well plate.
3. Add 25ul H_2O to 2 wells of the plate to serve as negative controls.
4. Add 25ul template and gene-specific primer mix to appropriate wells.

5. Seal all wells with strip caps.
6. Plates can be stored at 4°C for up to 48 hours (maybe more) before cycling.
7. Run PCR using program TKB (95° for 5min, 95° for 15s, 50° for 30s, 72° for 30s, go to step of 95° for 15s and repeat 34 times, 72° for 10min., 4° until PCR needed for subsequent steps
8. Run product on 1.5% agarose gel and check insert size. (Only need to check 1 well of each "gene.")
9. Clean PCR products using any commercially available kit for cleaning PCR products.

Gene purification

The ArrayIt™ kit from TeleChem, International, Inc. Sunnyvale, CA was used for gene purification. The following protocol was used:

1. Position a SuperFilter 100 on a 96-well vacuum manifold. Make sure the SuperFilter is properly fitted to allow a tight seal for vacuum filtration.
2. Add 500 µl of ArrayIt™ Binding Buffer to each well of the SuperFilter 100 using a 12-channel pipetting device set for 500 µl. Pipetting should be performed as quickly as possible (within 1 minute per plate) to minimize the loss of the Binding Buffer due to gravity flow. Avoid splashing the contents from well to well.
3. Quickly add 100 µl per well of PCR sample for a 96-well plate to the coreesponding well of the SuperFilter 100. Transfer the PCR samples to the SuperFilter 100 as quickly as possible (within 1 minute per plate) to minimize the loss of the Binding Buffer due to gravity flow.
4. Immediately mix the Binding Buffer and the PCR sample thoroughly by pipetting up and down 10 times with an automatic pipetting device. Mixing should be completed as quickly as possible (within 5 minutes after adding the Binding Buffer to the SuperFilter

100) to minimize the loss of the Binding Buffer due to gravity flow. void splashing the contents from well to well.

5. Apply a gentle vacuum such that a little trickle flows from the SuperFilter 100 to allowing binding of the PCR product to the SuperFilter 100 membrane. Primers, nucleotides, single-stranded products, salts, and other impurities pass through the SuperFilter 100 into the waste reservoir at the bottom of the vacuum filtration block.

6. Shut off the vacuum and add 800 µl of Wash Buffer to eachwell of the SuperFilter 100 with a 12-channel pipetting device. Apply a gentle vacuum until all of the Wash Buffer has passed through the SuperFilter 100 membrane. The 800 µl of Wash Buffer used in the first wash step is necessary to remove Binding Buffer and PCR sample that adheres to the walls of the SuperFilter 100 during mixing.

7. Shut off the vacuum and add 100 µl per well of Wash Buffer to the SuperFilter 100 with a 12-channel pipetting device. Apply a gentle vacuum until all of the Wash Buffer has passed through the membrane. Repeat this step with an additional 100 µl of Wash Buffer. The second and third wash steps remove additional trace contaminants from the bound PCR Product.

8. Apply a full vacuum for 3 minutes to dry the SuperFilter membrane. This removes small amounts of Wash Buffer that may interfere with the elution step and assists in fixing the DNA to the filter prior to elution.

9. Remove the SuperFilter 100 from the vacuum manifold and place it on an unmarked 96-well microplate.

10. Centrifuge the two plates for 5 minutes at ambient temperature in a microplate centrifuge (~500xg) to remove trace amounts of Wash Buffer. This step aids in eluting the DNA from the SuperFilter and improves yield.

11. Discard the unmarked microplate containing the residual wash Buffer.

12. Transfer the SuperFilter 100 containing the bound PCR product onto a marked 96-well microplate.

13. Re-hydrate the ArrayIt™ SuperFilter by adding 75 µl per well of H_20 (ph=8.0) with an automatic pipetting device. For maximal DNA recovery, be sure to add the 0.1X TE directly onto the surface of the SuperFilter membrane. The mild elution buffer (1mM TrisCl, 0.1 mM EDTA) is used to minimize the interference of the buffer in downstream applications.

Attaching hypersensitivity relevant genes to glass slide

The genes to be attached to the glass slides are amplified as provided herein. An important modification to the amplification process is the inclusion of amine primers, which can be obtained from any commercial source, i.e. Synthegen, such that a reactive amine group, a derivative thereof, or another reactive group is included in the amplified product. The amplified product is purified by any number of methods disclosed herein and immobilized or "spotted" onto a solid substrate, such as a glass slide, which can react with the amine group on the amplified product and form a covalent linkage.

An MD Generation II Array Spotter main instrument (Molecular Dynamics, 928 East Arques Avenue, Sunnyvale CA 04-86-4520) was used for spotting the hypersensitive genes according to following parameters:

MD ARRAY SPOTTER OPERATION

The terminology and equipment used in this example comprised the following:

Spotter:	MD Generation II Array Spotter main instrument
Spotting Chamber:	Area of spotter enclosed in glass which houses the pins, plates, trays and most spotter machinery.
Controller:	Dedicated Dell Computer and Monitor to right of Spotter Unit
Pins:	(6) fine tubes in the Spotter Unit which pick-up and spot the Target
Slides:	Std. size glass microscope slides with a special coating on one side
Plates:	Plastic 96 well plates which hold the Target solution to be spotted
Target:	A solution of PCR product which the spotter deposits on the slides.
N2 Tank:	5 ft. high steel gas tank labeled "Nitrogen, Compressed"
N2 :	The N2 gas from the N2 tank
Air Conditioner:	Kenmore air conditioner installed in window of spotting chamber
Humidifier 1:	Essick 2000 Evaporative Cooler against the window

Humidifier 2:	Bemis Airflow with white flexible duck into the Spotter Unit
Humidifier 3:	Bemis Airflow against the wall
Humidifier 4:	Kenmore QuietComfort 7
Vacuum Pump:	Gast Laboratory Oilless Piston Vacuum Pump
Dampbox:	The plastic sealable container containing an NaCl / water slurry

Materials used for reagent solutions are: Nanopure water, 0.2 M KCl (1/10 dilution of Stock 2M KCL in water), and 95% EtOH Reagent. The temperature control is adjusted to 60°. The spotter chambers are adjusted to be greater than 39 % relative humidity and less than 65° C. The spotting pins are pre-washed for 20 cycles.

Slide Preparation/Loading:

When the pre-wash is completed, the slides are first each blown with N_2 gas for about 2 seconds per side. The slides are inserted into the Spotter following Array Spotter Run Values. The slides are aligned using a clean narrow rod orienting it on the center right edge of the slide and gently pushed to the left until the slide is aligned vertically against the metal pins. After slides are loaded and straightened, a visual check is done to make sure no more debris had fallen. The humidity is confirmed to be greater than 39% relative humidity. The MD spotter recognizes 16 plates as a maximum for a run and will pause automatically after 8 plates. The MD spotter also advances sequentially to plates in an invariable order and is not programmable to accommodate unique plate sourcing scheme. Therefore, it is important to manually rotate (or shuffle) plates to accomplish the spotting for the canine arrays.

Blocking (Slide Preparation post-spotting)

This blocking procedure is important because it reduces the non-specific background signals. The amounts provided in this protocol are for 19 slides, however, a skilled artisan may make modifications accordingly. More staining dishes and slide racks will be required if more than 19 slides are to be blocked. A clean glass container is obtained

and filled with Nanopure H20. The container is placed on a hot plate and heated to a high temperature. A blocking solution is made by adding 2.5 ml of 20% SDS to 500mL blocking solution bottle. The blocking solution is warmed in microwave for 2.5 minutes and checked to determine if the temperature had reached 50°C. If the temperature of the solution is not at yet 50°C, then the solution is warmed in the microwave at 10 second intervals until it reached the desired temperature. One staining dish is placed on an orbital shaker with 4x SSC solution and turned to an agitation speed of 75 rpm. Slides are placed in metal racks and placed in boiling water for several minutes (i.e. 2 minutes). The slides are taken out of boiling water and allowed to cool briefly. The slides are then transferred to staining container containing 4x SSC solution on orbital shaker for several minutes (i.e. 2 minutes), rinsed with nanopure water in a staining container, and then briefly placed in blocking solution for about 15 minutes. After 15 minutes, the slides are taken out of the blocking solution and rinsed three times by dipping into three separate containers with nanopure water each time. The tops of the slides are dabbed lightly with a tissue and the slides are placed in a centrifuge for about 5 minutes at a speed of 1000 rpm.

Microarray RT Reaction

An exemplary procedure for labeling the probes is as follows. Fluorescence-labeled first strand cDNA probe is made from total or mRNA by first isolating RNA from control and treated cells, disclosed *supra*. This probe is hybridized to microarray slides spotted with DNA specific for hypersensitivity relevant genes. The materials needed to practice this example are: total or messenger RNA, primer, Superscript II buffer, dithiothreitol (DTT), nucleotide mix, Cy3 or Cy5 dye, Superscript II (RT), ammonium acetate, 70% EtOH, PCR machine, and ice.

The volume of each sample that would contain 20μg of total RNA (or 2μg of mRNA) is calculated. The amount of DEPC water needed to bring the total volume of each RNA sample to 14 μl is also calculated. If RNA is too dilute, the samples are concentrated

to a volume of less than 14 µl in a speedvac without heat. The speedvac must be capable of generating a vacuum of 0 Milli-Torr so that samples can freeze dry under these conditions. Sufficient volume of DEPC water is added to bring the total volume of each RNA sample to 14 µl. Each PCR tube is labeled with the name of the sample or control reaction. The appropriate volume of DEPC water and 8 µl of anchored oligo dT mix (stored at -20°C) is added to each tube.

Then the appropriate volume of each RNA sample is added to the labeled PCR tube. The samples are mixed by pipeting. The tubes are kept on ice until all samples are ready for the next step. It is preferable for the tubes to kept on ice until the next step is ready to proceed. The samples are incubated in a PCR machine for 10 minutes at 70°C followed by 4°C incubation period until the sample tubes are ready to be retrieved. The sample tubes are left at 4°C for at least 2 minutes.

The Cy dyes are light sensitive, so any solutions or samples containing Cy-dyes should be kept out of light as much as possible (i.e. cover with foil) after this point in the process. Sufficient amounts of Cy3 and Cy5 reverse transcription mix are prepared for one to two more reactions than would actually be run by scaling up the following protocols:

For labeling with Cy3
8 ul 5x First Strand Buffer for Superscript II
4 ul 0.1 M DTT
2 ul Nucleotide Mix
2 ul of 1:8 dilution of Cy3 (i.e., 0.125mM Cy3 dCTP).
2 ul Superscript II

For labeling with Cy5
8 ul 5x First Strand Buffer for Superscript II
4 ul 0.1 M DTT
2 ul Nucleotide Mix
2 ul of 1:10 dilution of Cy5 (i.e., 0.1mM Cy5 dCTP).
2 ul Superscript II

About 18 µl of the pink Cy3 mix is added to each treated sample and 18 µl of the blue Cy5 mix is added to each control sample. Each sample is mixed by pipeting. The samples are placed in a PCR machine for 2 hours at 45°C followed by 4°C until the sample

tubes are ready to be retrieved. The samples are transferred to Eppendorf tubes containing 600 µl of ethanol precipitation mixture. Some of the EtOH precipitation mixture is used to rinse the PCR tubes. The tubes are inverted to mix. Samples are placed in –80°C freezer for at least 20-30 minutes. If desired, samples may be left at -20°C overnight or over the weekend.

The samples are centrifuged for 15 minutes at 20800 x g (14000 rpm in Eppendorf model 5417C) and carefully the supernatant is decanted. A visible pellet is seen (pink/red for Cy3, blue for Cy5). It is a preferable to centrifuge the tubes at a fixed position so the pellet will be at a known area in the tube. In some rare instances, the probe is seen spread on one side of the tube instead of a tight pellet. If the pellet is white or nonexistent, the reaction has not occurred to maximal efficiency.

Ice cold 70% EtOH (about 1 ml per tube) is used to wash the tubes and the tubes are subsequently inverted to clean tube and pellet. The tubes are centrifuged for 10 minutes at 20800 x g (14000 rpm in Eppendorf model 5417C), then the supernatant is carefully decanted. The tubes are flash spun and any remaining EtOH is removed with a pipet. The tubes are air dried for about 5 to 10 minutes. protected from light. The length of drying time will depend on the natural humidity of the environment. For example, an environment in Santa Fe would require about 2 to 5 minutes of drying time. It is preferable that the pellet are not overdried.

When the pellets are dried, they are resuspended in 80 ul nanopure water. The cDNA/mRNA hybrid is denatured by heating for 5 minutes at 95°C in a heat block and flash spun.

To purify fluorescence-labeled first strand cDNA probes, the following materials are used: Millipore MAHV N45 96 well plate, v-bottom 96 well plate (Costar), Wizard DNA binding Resin, wide orifice pipette tips for 200 to 300 µl volumes, isopropanol, nanopure water. It is highly preferable to keep the plates aligned at all times during

centrifugation. Misaligned plates lead to sample cross contamination and/or sample loss. It is also important that plate carriers are seated properly in the centrifuge rotor.

The lid of a "Millipore MAHV N45" 96 well plate is labeled with the appropriate sample numbers. A blue gasket and waste plate (v-bottom 96 well) is attached. Wizard DNA Binding Resin (Promega cat#A1151) is shaken immediately prior to use for thorough resuspension. About 160 µl of Wizard DNA Binding Resin is added to each well of the filter plate that is used. If this is done with a multi-channel pipette, wide orifice pipette tips would have been used to prevent clogging. It is highly preferable not to touch or puncture the membrane of the filter plate with a pipette tip. Probes are added to the appropriate wells (80 µl cDNA samples) containing the Binding Resin. The reaction is mixed by pipeting up and down ~10 times. It is preferable to use regular, unfiltered pipette tips for this step. The plates are centrifuged at 2500 rpm for 5 minutes (Beckman GS-6 or equivalent) and then the filtrate is decanted. About 200 µl of 80% isopropanol is added, the plates are spun for 5 minutes at 2500 rpm, and the filtrate is discarded. Then the 80% isopropanol wash and spin step is repeated. The filter plate is placed on a clean collection plate (v-bottom 96 well) and 80 µl of Nanopure water, pH 8.0-8.5 is added. The pH is adjusted with NaOH. The filter plate is secured to the collection plate with tape to ensure that the plate did not slide during the final spin. The plate sat for 5 minutes and is centrifuged for 7 minutes at 2500 rpm. If there are replicates of samples they should be pooled.

To semi-quantitatively assess the incorporation of fluorescence into cDNA probes and to concentrate probes prior to hybridization, the following material is used: 384 well, 100 µl assay plate (Falcon Microtest cat#35-3980) and Wallac Victor 1420 Multilabel counter (or equivalent).

It is preferable that a consistent amount of cDNA is pipeted into the 384-well plate wells because readings will vary with volume. Controls or identical samples should be pooled at this step, if required. The probes are transferred from the Millipore 96 well plate to every other well of a 384 well assay plate (Falcon Microtest). This is done using a multi-

channel pipette. For replicate samples that have been pooled, 60 µl aliquots are transferred into wells of the assay plate.

The Cy-3 and Cy-5 fluorescence is analyzed using the Wallac 1420 workstation programmed for reading Cy3-Cy-5 in the 384-well format and the data is saved to disk. The typical range for Cy-3 (20µg) is 250-700,000 fluorescence units. The typical range for Cy-5 (20µg) is 100-250,000 fluorescence units. Settings for the Wallac 1420 fluorescence analyzer are as follows:

Cy3
CW lamp energy = 30445
Lamp filter = P550 slot B3
Emission filter= D572 dysprosium slot A4
Emission aperture = normal
Count time = 0.1 s

Cy5
CW lamp energy = 30445
Lamp filter = D642 samarium slot B7
Emission filter= D670 slot A8
Emission aperture = normal
Count time = 0.1 s

The dry-down process of the probes is as follows. Concentration of the cDNA probes is highly preferable so that they can be resuspended in hybridization buffer at the appropriate volume. The volume of the control cDNA (Cy-5) is measured and divide by the number of samples to determine the appropriate amount to add to each test cDNA (Cy-3). Eppendorf tubes are labeled for each test sample and the appropriate amount of control cDNA is allocated into each tube. The test samples (Cy-3) are added to the appropriate tubes. These tubes are placed in a speed-vac to dry down, with foil covering any windows on the speed vac. At this point, heat (45°C) may be used to expedite the drying process. Time will vary depending on the machinery. The drying process takes about one hour for 150 µl samples dried in the Savant. Samples may be saved in dried form at -20°C for up to 14 days.

To hybridize labeled cDNA probes to single stranded, covalently bound DNA target genes on glass slide microarrays, the following material are used: formamide, SSC, SDS, 2 μm syringe filter, salmon sperm DNA, hybridization chambers, incubator, coverslips, parafilm, heat blocks. It is preferable that the array is completely covered to ensure proper hybridization.

About 30 μl of hybridization buffer is prepared per sample. Slightly more than is what is needed should be made since about 100 μl can be lost during filtration.

Hybridization Buffer:	for 100 μl:
• 50% Formamide	50 μl formamide
• 5X SSC	25 μl 20X SSC
• 0.1% SDS	25 μl 0.4% SDS

The solution is filtered through 0.2 μm syringe filter, then the volume is measured. About 1 μl of salmon sperm DNA (10mg/ml) is added per 100 μl of buffer. Materials used for hybridization are: 2 Eppendorf tube racks, hybridization chambers (2 arrays per chamber), slides, coverslips, and parafilm. About 30 μl of nanopure water is added to each hybridization chamber. Slides and coverslips are cleaned using N_2 stream. About 30 μl of hybridization buffer is added to dried probe and vortexed gently for 5 seconds. The probe remained in the dark for 10-15 minutes at room temperature and then is gently vortexed for several seconds and then is flash spun in the microfuge. The probes are boiled for 5 minutes and centrifuged for 3 min at 20800 x g (14000 rpm, Eppendorf model 5417C). Probes are placed in 70 °C heat block. Each probe remained in this heat block until it is ready for hybridization.

Pipette 25 μl onto a coverslip. It is highly preferable to avoid the material at the bottom of the tube and to avoid generating air bubbles. This may mean leaving about 1 μl remaining in the pipette tip . The slide is gently lowered, face side down, onto the sample so that the coverslip covered that portion of the slide containing the array. Slides are

placed in a hybridization chamber (2 per chamber). The lid of the chamber is wrapped with parafilm and the slides are placed in a 42°C humidity chamber in a 42°C incubator . It is preferable to not let probes or slides sit at room temperature for long periods. The slides are incubated for 18-24 hours.

To obtain single stranded cDNA probes on the array, all non-specifically bound cDNA probe should be removed from the array. Removal of all non-specifically bound cDNA probe is accomplished by washing the array and using the following materials: slide holder, glass washing dish, SSC, SDS, and nanopure water. It is highly preferable that great caution be used with the standard wash conditions as deviations can greatly affect data.

Six glass buffer chambers and glass slide holders are set up with 2X SSC buffer heated to 30-34°C and used to fill up glass dish to 3/4th of volume or enough to submerge the microarrays. It is important to exercise caution in heating of the 2X SSC buffer since a temperature of greater than 35°C might strip off the probes. The slides are removed from chamber and placed in glass slide holders. It is preferable that the slides are not allowed dry out. The slides are placed in 2X SSC buffer but it is recommended that no more than 4 slides be placed per dish. Coverslips should fall off within 2 to 4 minutes. In the event that the coverslips do not fall off within 2 to 4 minutes, very gentle agitation may be administered. The stainless steel slide carriers are placed in the second dish and filled with 2X SSC, 0.1%SDS. Then the slides are removed from glass slide holders and placed in the stainless steel holders submerged in 2X SSC, 0.1%SDS and soaked for 5 minutes. The slides are transferred in the stainless steel slide carrier into the next glass dish containing 0.1X SSC and 0.1%SDS for 5 minutes. Then the slides are transferred in the stainless steel carrier to the next glass dish containing only 0.1X SSC for 5 minutes. The slides, still in the slide carrier, is transferred into nanopure water (18 megaohms) for 1 minute.

To dry the slides, the stainless steel slide carriers are placed on micro-carrier plates with a folded paper towel underneath. The top of the slides are gently dabbed with a tissue.

Then the slides are spun in a centrifuge (Beckman GS-6 or equivalent) for 5 minutes at 1000 rpm. It is very important that the slides do not air dry, as this will lead to increased background.

When the examples are practiced by a skilled artisan as disclosed, an analysis of a toxicological response to an agent, for example, cadmium chloride, can be obtained.

Preparation of cDNA

The following materials were used to prepare cDNA from RNA: total or messenger RNA; 3DNA™ Submicro™ Expression Array Detection Kit (Genisphere 3DNA 14 Phillips Parkway Montvale, NJ 07645; Kit numbers: K20F00-41 and K20F00-31); Linear Acrylamide (Ambion); RNase free water (Ambion); 0.5M NaOH/50mM EDTA;1M Tris-HDl, pH 7.5; 10mM Tris pH 8/1mM EDTA; 3M Ammonium Acetate; 70% Ethanol (Aldrich); 100% Ethanol (Aldrich); Denhardt's Salmon Sperm DNA (Sigma); RNase Zap (Ambion); Thermal Cycler; -80°C Freezer; Heat block; 4°C Microfuge; SpeedVac; MicroArray slides; Coverslips; Hybridization Chamber; 42°C Humidity Chamber; Parafilm.

For synthesis of cDNA, prepare 2 separate identical reactions for each sample. In a PCR or 1.5ml tube combine: 1.5ug lymphocyte RNA in 7ul DEPC treated water (if sample is too dilute, concentrate it in the SpeedVac at room temperature), and 3ul RT Primer. Separate tubes for treated and untreated RNA. Heat mixture to 80°C for 10 minutes, 4°C for 2 minutes. Place samples on ice and add the following: 4ul 5X RT buffer, 1ul dNTP mix, 4ul RNase free water, and 1ul Reverse transcriptase enzyme. Gently mix and centrifuge the contents of the tube. Incubate at 42°C for 1.5 to 2 hours. Stop the reaction by adding 3.5ul of 0.5M NaOH/50nM EDTA. Incubate at 65°C for 10 minutes to denature the DNA/RNA hybrids. Neutralize the reaction with 5ul of 1M Tris-HCL, pH 7.5. Transfer to 1.5ml tube if in PCR tube and add 38.5ul of 10mM Tris, pH8/1mM EDTA. Precipitate by adding the following to each tube: 4ul Linear acrylamide, 175ul 3M

Ammonium Acetate, and 625ul 100% Ethanol. Incubate at -80°C for 30 minutes. Centrifuge at 13,000 rpm in 4°C centrifuge for 15 minutes. Carefully decant supernatant.

11. Taqman® RT Reaction

Taqman® technology from Roche Molecular System was used in the following manner. The mRNA was converted to cDNA using 3μg total RNA and 1.5μl random hexamer primers. After a 10 minute incubation at 70°C the following components were added to the reaction mixture: 6μl of 5x first strand buffer, 3μl 0.1 DTT, 1.5μl 10mM dNTPs, 1.5μl Superscript enzyme and 6.5μl DEPC-treated water. The reaction is incubated for two hours at 45°C and 1μl of this reaction is used for the Taqman® assay. For the Taqman® assay 50μl reactions were set up with Rnase-free water, Taqman® Universal PCR Master Mix, target and control primers /probes and cDNA.

Real time PCR can be performed using the Taqman® assay . The method measures PCR product accumulation with a dual-labeled fluorogenic probe. The probes are labeled with 6-FAM on the 5' end and TAMRA on the 3' end. TAMRA is a quencher dye. This assay exploits the 5'-3' exonuclease property of Taq polymerase. When the probe hybridizes to its target the reporter dye (FAM) is cleaved by the 5' exonuclease activity of the Taq polymerase and can emit a fluorescent signal. With increasing cycles of amplification more signal is emitted and detected using an ABI 7700 sequence detector. For each gene, a set of two primers and a fluorogenic probe are designed and synthesized. For quantitation of mRNA the best design for probes and primers requires primers to be positioned over exon-intron junctions. This rules out amplification of contaminating genomic DNA. For initial studies, primer and probe sets have been designed for 13 genes that were up- or down-regulated by penicillin in differential display experiments. The probes and primer sets were tested for their ability to amplify genomic DNA. If genomic DNA was amplified, the probes and primers for that particular gene were not used for the Taqman® assay. Figure 9 and 10 show results obtained with a penicillin sensitive person

as well as a penicillin refractive person. The genes in these figures are as follows: 1A is Inhibitor of apoptosis protein-1, 76B is cyclin D2, 142B is Fc-gamma-receptorIIA (FCGR2A), 167B is chromosome 16 clone, RP11-296I10 198A is ribosomal protein S24 (RPS24a), 198B is ribosomal protein S24 (RPS24a). The Y-axis refers to levels of gene expression based on ABI Prism 7700 Realitive Quantification Software, in which cDNA levels are measured based on Ct (Cycle Threshold) values between control and treated samples.

Example8: Differential Protein Expression in Penicillin Treated and Untreated Human Lymphocytes from Penicillin Sensitive and Refractive Individuals

Protein expression in lymphocytes was studied using two technologies, SDS Polyacrylamide Electrophoresis (SDS-PAGE) and Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (SELDI-TOF) of proteins applied to ProteinChips. Differences in protein profiles, treated and untreated, for sensitive and refractive samples were observed using both techniques. The following methods were used:

Cell Preparation

For these experiments, blood was drawn from four refractive (control) individuals and two penicillin-sensitive individuals. White blood cells were isolated and cultured for 24 hours, using standard cell culture conditions. The cultures were split, half the cells were treated with penicillin, and all cells were grown for an additional 24 hours. Media was removed by centrifugation. Cells were then subjected to hypotonic lysis in nanopure water, followed by centrifugation to remove solid cellular debris. The supernatants were frozen prior to protein experiments. Cell lysates were concentrated by vacuum centrifugation prior to SDS-PAGE and ProteinChip experiments.

SDS-PAGE

Proteins were electrophoresed using a Bio-Rad MiniProtean gel apparatus, on ReadyGel Precast 4-20% acrylamide gels, using the standard method of Laemmli. For each concentrated lysate, 20 ul sample was mixed with 5 ul 5X SDS sample buffer. The samples were boiled for 10 minutes in the presence of 2-mercaptoethanol and half of each sample was loaded into corresponding wells on two identical gels. Two stains were used to visualize proteins in the replicate gels, Coomassie Blue and Ruby SYPRO (BioRad). Bands were observed directly for Coomassie stained gels, and by fluorescence scanning (Hitachi Scanner) for Ruby stained gels. All gels were dried in cellophane membranes as permanent records stored in (the laboratory notebook).

ProteinChip/SELDI-TOF

ProteinChips were obtained from Ciphergen Biosystems. Chips containing spots with hydrophobic (H4) and normal phase (NP) chromatographic surfaces were used. For the H4 surface, 1 ul acetonitrile was pipetted onto each spot to pre-wet the C-18 surface. Nanopure water was used to to wet the normal phase chip. Three microliters of concentrated lysate was added to each spot on replicate chips, with eight spots/samples per chip. The spots were dried at room temperature, then washed with 10% acetonitrile and nanopure water, for the H4 and NP chips, respectively. Washes were performed by pipetting 5 ul wash solution onto each spot, allowing a 5 minute incubation to resolubilize non-specifically bound biomolecules, and pipetting in and out five times prior to removing the wash buffer. Spots were dried under a 100 Watt bulb (placed 2 feet above benchtop). Each spot was then treated with 0.5 ul sinapinic acid (saturated in 50% acetonitrile, 0.5% trifluoroacetic acid), which acts as an energy absorbing "matrix" to assist laser ionization of proteins. Proteins were detected directly from the chips using a PBS-II mass spectrometer (Ciphergen Biosystems). Spectra were electronically stored in powerpoint files.

Results

Using both techniques, differences were observed in the protein profiles of treated and untreated, sensitive and refractive samples. The SDS-PAGE 1-D data is low resolution, but clearly shows increased production of at least four proteins in penicillin-treated sensitive cells, compared with the controls. Sensitivity was comparable for SELDI-TOF on ProteinChips is a more sensitive technique, and showed hundreds of peaks in each profile. The differences in protein spectra were striking, showing that refractive cells exhibit protein induction that is different than the induction in sensitive cells. While many differences were observed (at least 5-10 proteins), the similarities in the overall profiles was striking, and permits reasonable difference comparison by providing internal standards.

TABLE 1

Generic Name
acetaminophen
acetaminophen/codeine
acetohydroxamic acid
actinomycin D
acyclovir
adenosine
albuterol
alendronate
alendronate sodium
alglucerase
allopurinol
alosetron
alprazolam
alprostadil
alteplase
ambenonium
amifostine
amiloride
aminobenzoate potassium
aminoglutethimide
aminopurine
aminosalicylate sodium
amiodorone
amitriptyline
amlodipine
amoxapine
amoxicillin
amphetamine mixed salts
ampicillin
amprenavir
amyl nitrite
anagrelide
ancrod
androgens
anistreplase
anthralin
araC
aspirin
aspirin
astemizole
atenolol
atorvastatin

atovaquone
atropine
attapulgite
azathioprine
azelastine
azithromycin
aztreonam
bacampicillin
baclofen
beclomethasone
belladonna
benazepril
benazepril
benzodiazepines
benzoyl peroxide
benztropine
beta carotene
betamethasone
betamethasone
betamethasone valerate
bethanechol
bisacodyl
bismuth subsalicylate plus
bisoprolol/HCTZ
bleomycin
bradykinin antagonist
bromfenac
brominide tartrate
bromocriptine
bronchodilators
buclizine
budesonide
bumetanide
bupropion HCL
buspirone
busulfan
calcipotriene
calcitonin salmon
calfactant
candesartan cilexetil
capsaicin
captopril
carbamazapine
carbenicillin
carbidopa
carboplatin

carisoprodol
carmustine
carvedilol
cefaclor
cefepime
cefprozil
ceftibuten
cefuroxime
celecoxib
cephalexin
cephalosporins
cerivastatin
cetirizine
chenodiol
chlophedianol
chloral hydrate
chlorambucil
chloramphenicol
chloroquine
chlorpropamide
chlorthalidone
chlorzoxazone
cholestyramine
cimetidine
cinoxacin
ciprofloxacin
(+)-cis-3,5-dimethyl-2-(3-pyridyl)thiazolodin-4
cisapride
cisplatin
citalopram
clarithromycin
clavulanate
clavulanate
clavulanic acid
clidinium
clindamycin
clofibrate
clomiphene
clonazepam
clonidine
clotrimoxazole
cloxacillin
clozapine
codeine
colchicine

colestipol
collagen-alginate
conjugated estrogens
copolymer-1
cortisone
courmarin
cromolyn
cyclacillin
cyclandelate
cyclizine
cyclobenzaprine
cyclopegic
cyclopentolate
cyclophosphamide
cycloserine
cyclosporine
cyclosporine A
cytoxin
dalteparin injection
danazol
dantrolene
dapsone
daunomycin
daunorubicin
dehydrocholic acid
desmopressin
desogestrel
dexamethasome
dextromethorphan
dextrothyroxine
diazepam
diazoxon
dichloralphenazone
diclofenac
diclofenacdihydrazine
dicloxacillin
dicyclomine
didanosine
difenoxin
digitalis glycosides
digoxin
dihydrazine
dihydroergotamine mesylate
dihydrolazine
diltiazem
dimethyl sulfoxide

dinoprostone
dione
diphenidol
diphenoxylate
dipyridamole
dipyridamole
disopyramide
disulfiram
divalproex
divalproex sodium
docusate sodium
dolasetron mesylate
donepezil
doxazosin
doxercalciferol
doxorubicin
doxycycline
enalapril
enoxaparin
entacapone
ephedrine
epirubicin
eptifibatide
ergoloid mesylates
ergonovine
erythromycin
estradiol
estramustine
etanercept
ethacrynic acid
ethchlorvynol
ethinamate
ethinyl estradiol
ethinyl estradiol
ethionamide
etidronate
etoposide
etretinate
exemestane
famciclovir
famotidine
felbamate
felodipine
felodipine SR
fenofibrate
fenoldopam mesylate

fentanyl citrate
fexofenadine
fialuridine
finasteride
flavoxate
flecainide acetate
flosequinan
fluconazole
flunisolide
fluoroquinolones
fluorouracil
fluoxetine
flutamide
fluticasone
fluticasone
fluticasone propionate
fluvastatin
fluvoxamine maleate
foscarnet sodium
fosinopril
fosphenytoin
furazolidone
furosemide
gabapentin
ganciclovir
ganirelix acetate
gemcitabine
gemfibrozil
glimepiride
glipizide
glucagon
glyburide
glycopyrrolate
gold compounds
gold sodium thiomalate
granisetron
grepafloxacin
griseofulvin
guaifenesin
guanabenz
guanadrel
guanethidine
guanfacine
haloperidol
heparin
hismanol

hydantoin
hydralazine
hydrochlorothiazide
hydrocodone
hydrocortisone
hydroxychloroquine
hydroxyurea
hydroxyzine
hyoscine
hyoscyamine
hyoscyamine
hyperozia
ibuprofen
ibutilide fumarate
imiglucerase injection
imiquimod 5% cream
inactivated hepatitis A vaccine
indapamide
indinavir
indomethacin
insulin
interferon-beta-1a (recombinant)
interferon-beta-1b (recombinant)
iodinated glycerol
iodoquinol
ipecac
iphosphamide
ipratropium
irbesartan
irinotecan
isometheptene
isoniazid
isoproterenol
isosorbide mononitrate S.A.
isotretinoin
isoxsuprine
isradipine
itraconazole
kanamycin
ketoconazole
ketorolac
lactulose
lamivudine, 3TC
lamotrigine
lansoprazole
latamoxef

latanoprost
leflunomide
letrozole
leucovorin
leuprolide
levamisole
levetiracetam
levobupivacaine
levocabastine
levocarnitine
levodopa
levofloxacin
levonorgestrel
levothyroxine
lidocaine
lincomycin
liposomal amphotericin B
lisinopril
lispro insulin
lithium
l-norgestrel
l-norgestrel/ethinyl estradiol
lomustine
loperamide
loracarbef
loratadine
Loratidine/Pseudoephedrine
lorazepam
losartan
lovastatin
loxapine
magnesium sulfate
maprotiline
masoprocol
mazindol
mecamylamine
mechlorethamine
meclizine
medroxyprogesterone
medroxyprogesterone
mefloquine
melatonin
melphalan
menotropin
meprobamate
merbarone

mercaptopurine
meropenem
mesalamine
metformin
methenamine
methicillin
methotrexate
methylcellulose
methyldopa
methylergonovine
methylphenidate
methylprednisolone
methyprylon
methysergide
metoclopramide
metoprolol
metoprolol
metronidazole
metyrapone
metyrosine
mexiletine
mibefradil
miconazole cream 2%
miglitol
minocycline
minoxidil
misoprostol
misoprostol
mitotane
mixed amphetamines
moclobemide
molindone
mometasone
moricizine
moxifloxacin
mupirocin
nabilone
nabumetone
nafarelin
nafcillin
nalidixic acid
naltrexone
naproxen
naratriptan
natamycin
navirapine

nedocromil
nefazodone
neomycin
Neomycin/Polymx/HC
neostigmine
nicardipine
nicorandil
nicotine
nifedipine
nimodipine
nitrofurantoin
nitroglycerin
nizatidine
norethindrone
norethindrone/ ethinyl estradiol
norgestimate
norgestimate/ethinyl estradiol
norgestrel
norgestrel/ethinyl estradiol
nylidrin
nystatin
ofloxacin
olsalazine
omeprazole
orphenadrine
oxacillin
oxaprozin
oxtriphylline
oxybutynin
oxycodone
oxymetazoline
paclitaxel
pancreatin
pancrelipase
papaverine
paraldehyde
paramethasone
paregoric
paroxetine
pediculisides
pemoline
penicillamine
penicillin
pentamidine
pentoxifylline
pepsin

pergolide
perhexiline
perindopril
perphenazine
pexiganan acetate
phenazopyridine
phendimetrazine
phenformin
phenobarbital
phenolphthalein
phenothiazines
phentermine
phenylephrine
phenylephrine
phenylpropanolamine
phenylpropanolamine
phenytoin
pilocarpine
pioglitazone
piroxicam
podophyllum
poloxamer 188
polycarbophil calcium
polyethylene glycol
polythiazide
potassium chloride
potassium iodide
potassium phosphates
pramipexole
pravastatin
prazosin
prednisolone
prednisone
primaquine
primethamine
primidone
probenecid
probucol
procainamide
procarbazine
progestins
promethazine
propafenone
propantheline
propoxyphene
propranolol

propulsid
pseudoephedrine
psoralens
psyllium
pyridostigmine
pyridoxine (vitamin b-6)
quinacrine
quinapril
quinidine
quinine
rabeprazole
raloxifene
ramipril
ranitidine
recombinant clotting factor VIII
recombinant interferon alpha-2b
recombinant OspA
remoxipide
reserpine
rezulin
ribavirin
rifampin
rimantadine
risedronate
risperidone
ritodrine
rosiglitazone
salicylates
salmeterol
saquinavir
scopolamine
seldane
selegiline
sertraline
sibutramine
sildenafil citrate
simethicone
simvastatin
s-mephenytoin
sodium ferric gluconate
soman
somatostatin
sotalol
spironolactone
stanol esters
streptozotocin

succinimide
sucralfate
sulfacytine
sulfadoxine
sulfamethoxazole
sulfamethoxazole
sulfasalazine
sulfinpyrazone
sulfisoxazole
sumatriptan
(s)-warfarin
tacrine
tamoxifen
tamsulosin
telmisartan
temazepam
terazosin
terbinafine HCl
terbutaline sulfate
terfenadine
terpin hydrate
testolactone
tetracycline HCl
tetracyclines
theophylline
thiamine
thiazide
thioguanine
thiopurine
thiothixene
tiagabine
ticlopidine
tienilic acid
timolol
tiopronin
tirofiban
tobramycin
tobramycin/dexamethasone
tocainide
tolbumamide
tolcapone
tolterodine
topotecan
toremifene
tramadol
trandolapril

trastuzumab
trazodone
tretinoin
triamcinolone
triamterene/HCTZ
triamterine
triamterine
triazolam
trihexyphenidyl
trilostane
trimeth/sulfameth
trimethobenzamide
trimethoprim
troglitazone
trovafloxacin
urokinase
ursodiol
valproic acid
valsartan
vancomycin
venlafaxine
verapamil
vincristine
warfarin
xanthine
xylometazoline
zafirlukast
zalcitabine
zidovudine
zolpidem

TABLE 2

Industrial Chemicals
1,2-Dibromomethane
2,4-dinitrotoluene
2-methylpentane
3-methylpentane
4,4'-methylene bis
7, 12-dimethylbenz[a]anthracene
Acetone
Acrylamide
Acrylonitrile
Apha methylstyrene
Aluminum
Aniline
Antimony
Arsenic
Barium
Baygon
Benzene
Benzidine
Berylium
Bta-napthylamine
Biphenyl
Cadmium
Carbamate(s)
Carbaryl
Carbon disulfide
Carbon monoxide
Carbon tetrachloride
Chloroform
Chromium VI
Cobalt
Copper
Cumene
Cyanamide
Cyanides
Cyclohexane
Cyclohexanone
Cyclophosphamide
DDT
DEHP
Dichlorobenzene
Dichloromethane

Dieldrin
Diethylamine
Diethystilbesterol
Dimethylacetamide
Dimethylformamide
Dinitroorthocresol
Dioxane
Endrin
Enflurane
Ethylbenzene
Ethylene oxide
Ethyleneglycol dinitrate
Ethyleneglycol(s)
Fluoride
Furfural
Furfuryl alcohol
Germanium
Halothane
Hexachlorobenzene
Hexachlorobutadiene
Isopropanol
Isopropylnitrate
Lead
Lead tetraethyl
Lindane
Maleic anhydride
Manganese
Mercury
Methanol
Methylchloride
Methylethylketone
Methylmercury
Monobromomethane
Monochlorobenzene
n-hexane
Nickel
Nitrobenzene
Nitroglycerine
Nitrous oxide
Organophosphorus
Parathione
Pentachlorophenol
Phenol
Phtalic anhydride
Polychlorinated biphenyl
Polycyclic hydrocarbons

Propyleneglycol
Selenium
Silver
Stryrene
Synthetic pyrethroids
T-butylhydroperoxide
TCDD
Tellerium
Tert-butylphenol
Tetrachloroethylene
Thalium
Toluene
Toluene diisocyanate
Trichloroethane
Trichloroethylene
Triethylamine
Triethylbenzenes
Uranium
Vanadium
Vinyl chloride
Xylene
Zinc

TABLE 3

Gene Name	Genbank Accession No.
Ataxia telangeictasia	U33841
ATF4 (activating trxn factor 4)	D90209
ATP-dep. Helicase II (70kDa)	M32865
ATP-dep. Helicase II (Ku80)	M30938
Bax (alpha)	L22473
Bcl-xL	Z23115
c-Abl	M14752
c-Fos	K00650
Chk1	AF016582
c-H-Ras	J00277
c-Jun	J04111
Clusterin (serum protein 40)	X14723
c-Myc	X00364
Connexin 32 (gap junction protein)	X04325
Cyclin G	D78341
Cytochrome P-1-450 (cyp1A1)	K03191
DNA binding protein inhibitor ID-2	D13891
DNA dependent helicase	L36140
DNA dependent protein kinase	U47077
DNA ligase IV	X83441
DNA polymerase alpha	X06745
DNA repair protein (Rad 50)	U63139
DNA repair protein XRCC1	M36089
DNA topoisomerase I	J03250
ERCC1 (excision repair protein)	M13194
DNA repair helicase II ERCC-3	M31899
Excision repair ERCC-5	L20046
Gadd153	S40706
Gadd45	M60974
Glutathione Peroxidase	M21304
HDLC1	U32944
Hsp70	M11717
Hsp90	X15183
ICE Rel II	U28014
Mdm-2	U33199
Mdr-1	M14758
MnSOD	Y00985
Mut S homologue (hMSH2)	U04045
MUTL homolog=hMLH1	U07418
Poly (ADP-ribose) polymerase (PARP)	M32721/X56140
Prolifer.cell nuclear antigen (PCNA)	J04718
RAD	L24564

RAD51 homolog	D13804
RNA-dependent Helicase (DEAD-box protein p72)	U59321
SQM1	M33374
Stress activated protein kinase JNK1	L26318
UV Excision repair protein RAD23 (XP-C)	D21090
Vascular cell adhesion molecule 1 (VCAM-1)	M73255
Alpha-Tubulin	K00558
Beta-Actin	X00351
Glucose-6-phosphate dehydrogenase (G6PD)	X03674
cytochrome p-450 4A	L04751
connexin 40	L34954
Bak	U16811
Collagenase, type I interstitial	X54925
G/T mismatch binding protein	U28946
Mismatch repair/binding protein (hMSH3)	U61981
DNA mismatch repair protein (hPMS2)	U14658
Apolipoprotein A-II	M29882
Acyl CoA dehydrogenase	U12778
Carnitine palmitoyl CoA transferase	M58581
Hepatic lipase	J03540
Ornithine decarboxylase	M16650
Superoxide dismutase Cu/Zn (SOD)	K00065
Ref-1=redox factor	S43127
Thioredoxin	J04026
Glutathione synthetase	L42531
Glutathione reductase	X15722
Thymidine kinase	K02581
Bag-1=bcl-2	S83171
BRCA1	U14680
Phenol sulfotransferase	U26309
Aldehyde dehydrogenase 1 (ALDH-1)	K03000
Aldehyde dehydrogenase 2 (ALDH-2)	K03001
12-lipoxygenase	M58704
Phospholipase A2	M86400
Calnexin	M94859
Apolipoprotein CIII.	X01388
Branched chain Acyl-CoA Oxidase	X95190
Cyclin dependent kinase 4 (cdk4)	M14505
ERp72	J05016
MCL-1	L08246
HMG CoA reductase	M11058
Lipopolysaccharide binding protein	M35533
Lysyl oxidase	M94054
Farnesol Receptor	U68233
Osteopontin	J04765
P38 mitogen activated protein (MAP) kinase	L35253
Peroxisomal acyl-CoA oxidase	X71440

Uncoupling protein 2 (UCP2)	U82819
Very-long-chain acyl-CoA dehydrogenase	D43682
Vimentin	X56134
EGR1	X52541
GRP94	X15187
P53	K03199
Defender against cell death-1	D15057
Hypoxanthine-guanine phosphoribosyltransferase	V00530
Aspartate aminotransferase, mitochondrial	M22632
Creatine kinase B	L47647
Peroxisome assembly factor-1	M86852
T-cell cyclophilin	Y00052
Transferrin	M12530
UDP-glucuronosyltransferase 2B	AF016492
Octamer-binding protein 1	X13403
E-cadherin	L08599
Catalase	X04076
11 beta-hydroxysteroid dehydrogenase type II	U14631
Bilirubin UDP-glucuronosyltransferase isozyme 1	M57899
Calreticulin	M84739
Calcineurin-B	M30773
Catechol-O-methyltransferase	M58525
Fas antigen	M67454
DNA repair and recombination homologue (RAD52)	L33262
Flavin-containing monooxygenase 1	M64082
Gamma-glutamyl transpeptidase	L20490
Insulin-like growth factor binding protein 1	M31145
Oxygen-regulated protein 150	U65785
Thymidylate synthase	X02308
Biliverdin reductase	U34877
Adenine nucleotide translocator 1	J02966
Hepatocyte nuclear factor 4	X76930
RANTES	M21121
Phosphoglycerate kinase	V00572
PAPS synthetase	Y10387
Plasminogen activator inhibitor 2	M18082
Enolase alpha	M14328
Interferon inducible protein 15	M13755
Insulin-like growth factor I	M37484
Platelet/endothelial cell adhesion molecule-1	M28526
60S ribosomal protein L6	X69391
FosB	L49169
Alpha-catenin	D13866
FEN-1 (endonuclease)	L37374
GOS24 (zinc finger transcriptional regulator)	M92843
Caspase 8 (FLICE)	U58143
Caspase 3 (CPP32-beta)	U13738

Caspase 7 (Mch3-alpha)	U37448
Intercellular adhesion molecule-3	X69819
Phosphoenolpyruvate carboxykinase	X92720
Alpha-1 acid glycoprotein	M13692
IkB-a	M69043
Protein-tyrosine phosphatase	M83738
Ubiquitin conjugating enzyme (Rad6 homolog)	M74524
Alpha-2-macroglobulin	M11313
Zinc finger protein 37	AF022158
Cyclin-dependent kinase inhibitor p27kip1	U10906
Caspase 1	U13697
Organic anion transporter 1	AF057039
Alcohol dehydrogenase 2	M24317
Alchohol dehydrogenase 4	M15943
Annexin V	M21731
Calbindin-D (28kDa)	X06661
Colony-stimulating factor-1	M37435
Hypoxia-inducible factor 1 alpha	U22431
Growth arrest-specific protein 1	L13698
Inhibitor of apoptosis protein-1	AF070674
Nucleic acid binding protein	U19765
OX40 ligand	X79929
Retinoic acid receptor gamma 1	M38258
Cytochrome c oxidase subunit IV	M34600
Glutathione S-transferase theta-1	X79389
Survivin	NM001168
STAT 3	AJ012463
Growth arrest-specific protein 3	L03203
Cyclin D3	M92287
ID-1	X77956
Interleukin-1 beta	X02532
Interleukin-8	Y00787
Monocyte chemotactic protein-1	S69738
Phenylalanine hydroxylase	K03020
Prohibitin	S85655
Cathepsin L	M20496
Transthyretin	X59498
Stromelysin-1	X05232
Spermidine/spermine N1-acetyltransferase (SSAT)	M55580
Ferritin H-chain	L20941
Transferrin receptor	M11507
Ceruloplasmin	M13699
Glucosylceramide synthase	D50840
Leukemia inhibitory factor (LIF)	X13967
Integrin beta-1	X07979
Vascular endothelial growth factor receptor 1 (flt-1)	X51602
Urokinase plasminogen activator receptor (uPAR)	U08839

c-fms	X03663
c-erb B-2	X03363
C5a anaphylatoxin receptor	M62505
FYN proto-oncogene	NM002037
Peroxisomal enoyl-CoA hydratase: 3-hydroxyacyl-CoA dehydrogenase	L07077
Nucleoside diphosphate kinase beta isoform	X73066
Myelin basic protein	M13577
Peroxisomal 3-oxoacyl-CoA thiolase (=rat peroxisomal 3-ketoacyl-CoA thiolase 2)	X12966
Prostaglandin H synthase	S36271
Retinoid X receptor alpha	NM002957
Interleukin-13	X69079
Tryptophanyl-tRNA synthetase (WRS)	M61715
Silencer of death domains	AF111116
Mannose receptor	J05550
Death receptor 5 (DR5)	AF016268
Neomycin	U55761

TABLE 4

(clone hKvBeta3) K+ channel beta subunit
APO-1 cell surface antigen
11-beta hydroxysteroid dehydrogenase type II
12-lipoxygenase
17-beta hydroxysteroid dehydrogenase
25-hydroxyvitamin D3-1 alpha-hydroxylase
60S ribosomal protein L6
6-C-kine
6-O-methylguanine-DNA methyltransferase
acetylhydrolase IB beta-subunit
Acid ceramidase
actin-binding protein (filamin) (ABP-280)
Activating transcription factor 2
Activating transcription factor 3
Activating transcription factor 4
Activin beta E
Activin receptor type II
Acyl - CoA dehydrogenase
Acyl CoA Carrier Protein
Adenine nucleotide translocator 1
Adenylyl cyclase-associated protein (CAP)
Adhesion protein (SQM1)
Alanine aminotransferase
Alcohol dehydrogenase 1
Alcohol dehydrogenase 2
Alcohol dehydrogenase 3
Alcohol dehydrogenase 4
Alcohol dehydrogenase 7
Aldehyde dehydrogenase 1
Aldehyde dehydrogenase 2
Aldehyde dehydrogenase 3
Aldose reductase
Alpha 1-antitrypsin
Alpha 1-inhibitor III
Alpha interferon
Alpha(I)procollagen
Alpha-1 acid glycoprotein
Alpha-1 antichymotrypsin
Alpha-2 macroglobulin
Alpha-2 microglobulin
Alpha-catenin
Alpha-tubulin
Amyloid protein homologue
Androgen receptor
Annexin V

Antiquitin, 26g turgor protein homolog
Aorta caldesmon
APC gene
Apolipoprotein A1
Apolipoprotein AII
Apolipoprotein CIII
Apolipoprotein E
Aryl hydrocarbon receptor
Aspartate aminotransferase, mitochondrial
Ataxia telangeictasia
ATP Synthase 6
ATP-dependent helicase II (70kDa)
ATP-dependent helicase II (Ku80)
Atrial natiuretic factor
BAG-1
BAK
Bax (alpha)
Bcl-2
Bcl-3
Bcl-xL
Beta-actin
Beta-chemokine I-309
Bile salt export pump (sister of p-glycoprotein)
Biliary glycoprotein
Bilirubin UDP-glucuronosyltransferase isozyme 1
Biliverdin reductase
B-myb
Bone morphogenetic protein-4
Bone sialoprotein gene
Brain-derived neurotrophic factor
Branched chain acyl-CoA oxidase
BRCA1
BR-cadherin
Breast basic conserved gene (ribosomal protein L13)
Breast cancer resistance protein (BCRP)
C10 beta-chemokine
C4b-binding protein
C5a anaphylatoxin receptor
c-abl
Calbindin-D (28kDa)
Calbindin-D (9K)
Calcineurin-B
Calnexin
Calprotectin
Calreticulin
canalicular multispecific organic anion transporter

Carbonic Anhydrase III
Carcinoembryonic antigen (CD66e)
Carcinoembryonic antigen family member 2
cardiac gap junction protein
Carnitine palmitoyl-CoA transferase
Casein kinase 1 delta
Caspase 1
Caspase 2 (Nedd2)
Caspase 3 (CPP32-beta)
Caspase 5 (ICE rel-III)
Caspase 6 (Mch2-alpha)
Caspase 7 (Mch3-alpha)
Caspase 8 (FLICE)
Catalase
Catechol-O-methyltransferase
Cathepsin G
Cathepsin L
Caveolin-1
CCAAT/enhancer-binding protein alpha
CCAAT/enhancer-binding protein epsilon
CCR-5
CD44 (metastasis suppressor gene)
CD64 (Fc gamma)
Cell division cycle protein 2
Cell division cycle protein 25
Cellular retinoic acid binding protein 1
Cellular retinoic acid binding protein 2
c-erb B-2
c-erbA-1
Ceruloplasmin (ferroxidase)
c-fms (CSF-1 receptor)
c-fos
CHD2
Checkpoint kinase-1
Cholesterol esterase
c-H-ras
CIG49 (cig49)
c-jun
Clone 22 mRNA, alternative splice variant alpha-1
CLP
Clusterin
c-myb
c-myc binding protein
Collagen type II
Colony-stimulating factor-1
Complement component C3

Connexin 30
Connexin-32 (aka gap junction protein)
Connexin-40
Corticosteroid binding globulin
Corticotropin releasing hormone
C-reactive protein
Creatine kinase B
Csa-19
CTCF
CXCR4
Cyclin A1
Cyclin D1
Cyclin D3
Cyclin dependent kinase 1
Cyclin dependent kinase 2
Cyclin dependent kinase 4
Cyclin dependent kinase inhibitor 1A
Cyclin E
Cyclin G
Cyclin-dependent kinase 4 inhibitor B (P16)
Cyclin-dependent kinase inhibitor P27Kip1
Cyclooxygenase 2
Cysteine protease CPP32 isoform alpha
Cystic fibrosis transmembrane conductance regulator
Cytochrome c oxidase subunit III
Cytochrome c oxidase subunit IV
Cytochrome P450 11A1
Cytochrome P450 17A
Cytochrome P450 1A1
Cytochrome P450 1A2
Cytochrome P450 1B1
Cytochrome P450 2A1
Cytochrome P450 2A3
Cytochrome P450 2A6
Cytochrome P450 2B1
Cytochrome P450 2B10
Cytochrome P450 2B2
Cytochrome P450 2C11
Cytochrome P450 2C12
Cytochrome P450 2C19
Cytochrome P450 2C9
Cytochrome P450 2D6
Cytochrome P450 2E1
Cytochrome P450 2F2
Cytochrome P450 3A1
Cytochrome P450 3A4

Cytochrome P450 4A
Cytochrome P450 4A1
cytoskeletal gamma-actin
Damage-specific DNA binding protein p48 subunit
Death receptor 5 (DR5)
Defender against cell death-1
Deleted in colorectal cancer
Delta-like protein
Diacylglycerol kinase zeta
Dihydrofolate reductase
Disulfide isomerase related protein (ERp72)
DNA binding protein inhibitor ID2
DNA dependent helicase
DNA dependent protein kinase
DNA ligase I
DNA ligase III
DNA ligase IV
DNA mismatch repair protein (MLH1)
DNA mismatch repair protein (PMS2)
DNA mismatch repair/binding protein (MSH3)
DNA polymerase alpha
DNA polymerase beta
DNA repair and recombination homologue (RAD 52)
DNA repair helicase II ERCC-3
DNA repair protein (RAD 50)
DNA repair protein (XRCC1)
DNA replication factor C (36kDa)
DNA topoisomerase I
DNA topoisomerase II
DNA-binding protein (APRF)
DOC-2
Dopamine beta-hydroxylase
Dopamine receptor D2
DRA
Dynamin (DNM)
Dynein light chain 1
E2F-1
Early growth regulated protein 1
E-Cadherin
ECE-1 (endothelin converting enzyme)
ELAV-like neuronal protein-2 Hel-N2
Elongation factor 1-alpha 1 (PTI-1)
Endothelin-1
Enolase alpha
enteric smooth muscle gamma-actin

Eosinophil-derived neurotoxin
Eotaxin
Epidermal growth factor
Epoxide hydrolase
ERA-B
ERCC 1 (excision repair protein)
ERCC 3 (DNA repair helicase II)
ERCC 5 (excision repair protein)
ERCC 6 (excision repair protein)
Erythrocyte membrane protein
Erythropoietin
Erythropoietin receptor
E-Selectin
Estrogen receptor
Extracellular-signal-regulated kinase 1
Farnesol receptor
Fas antigen
Fas associated death domain (FADD)
Fas ligand
Fas/Apo1 receptor
Fatty acid synthase
Fatty acyl-CoA oxidase
Fatty acyl-CoA synthase
FEN-1 (endonuclease)
Ferritin H-chain
FGF-1
FGF-7
Fibrinogen gamma chain
Fibronectin receptor
FIC1
Filaggrin
Flavin containing monooxygenase 1
Flavin containing monooxygenase 3
for gamma-interferon inducible early response gene (with homology to platelet proteins)
FosB
Fra-1
Fucosyl transferase (alpha-1,2-fucosyltransferase)
Fyn proto-oncogene
Gadd153
Gadd45
Galanin
Gamma glutamylcysteinyl synthetase
Gamma-glutamyl hydrolase (hGH)
Gamma-glutamyl hydrolase precursor
Gamma-glutamyl transpeptidase

Garg-16
GAS-7
GCLR
GCLS
Gelsolin
Glucocorticoid receptor
Glucose-6-phosphate dehydrogenase
Glucose-regulated protein 170
Glucose-regulated protein 58
Glucose-regulated protein 78
Glucose-regulated protein 94
Glucosylceramide synthase
Glutamic-oxaloacetic transaminase
Glutamic-pyruvic transaminase
Glutamine synthetase
Glutaredoxin
Glutathione peroxidase
Glutathione reductase
Glutathione S-transferase alpha subunit
Glutathione S-transferase theta-1
Glutathione S-transferase Ya
Glutathione synthetase
Glyceraldehyde 3-phosphate dehydrogenase
Gonadotropin (alpha subunit)
GOS24 (zinc finger transcriptional regulator)
Granulin
Granulocyte-macrophage colony-stimulating factor
Growth arrest-specific protein 1
Growth arrest-specific protein 3
GT mismatch binding protein
Hamartin (TSC1)
H-cadherin
Heat shock protein 12
Heat shock protein 27
Heat shock protein 47
Heat shock protein 70
Heat shock protein 90
Helicase-like transcription factor
Heme binding protein 23
Heme oxygenase-1
Hemopexin
Hepatic lipase
Hepatocyte growth factor
Hepatocyte growth factor activator
Hepatocyte nuclear factor 4
Histamine N-methyltransferase

Histidine decarboxylase
Histone 2A
Histone 2B
Histone deacetylase 1 (HDAC-1)
hMEF2C, myocyte enhancer-binding factor 2
HMG CoA reductase
HMG-I protein isoform mRNA (HMGI gene), clone 7C
Hydroxysteroid sulfotransferase a
Hypoxanthine-guanine phosphoribosyltransferase
Hypoxia-inducible factor 1 alpha
ICE-rel II (Caspase 4)
ID-1
IkB-a
immunoglobulin lambda heavy chain
Immunophilin homolog ARA9
Inhibitor of apoptosis protein 1
Inhibitor of apoptosis protein 2
Insulin-like growth factor binding protein 1
Insulin-like growth factor binding protein 2
Insulin-like growth factor binding protein 5
Insulin-like growth factor binding protien 3
Insulin-like growth factor I
Insulin-like growth factor II
Integrin alpha
Integrin alpha L
Integrin beta1
Integrin beta2
Integrin beta-4
Intercellular adhesion molecule-1
Intercellular adhesion molecule-2
Intercellular adhesion molecule-3
Interferon gamma
Interferon inducible protein 10
Interferon inducible protein 15
Interferon stimulatory gene factor-3
Interleukin-1 alpha
Interleukin-1 beta
Interleukin-10
Interleukin-12
Interleukin-13
Interleukin-18
Interleukin-2
Interleukin-3
Interleukin-4
Interleukin-5

Interleukin-6
Interleukin-8
Involucrin
IRF-7
Iron permease (FTR1)
ISG-15
Jagged 1
Jagged 2
JNK1 stress activated protein kinase
JunB
JunD
K+ channel beta 2 subunit
KAI1 metastasis suppressor gene (CD82)
K-cadherin
Keratin 4
Keratin 6 isoform K6e (KRT6E)
Keratin K17
Keratinocyte growth factor
Ki67
Ku autoimmune antigen gene (p80)
L09604
Lactate Dehydrogenase-B
Lactoferrin
Leukemia inhibitory factor (LIF)
Lipopolysaccharide binding protein
Lipoprotein lipase precursor
Liposin
Liver fatty acid binding protein
L-myc
long-chain acyl-CoA synthetase
Low density lipoprotein receptor
Lung cancer antigen NY-LU-12 variant A
Luteinizing hormone
Lymphoid enhancer-binding factor-1 (LEF-1)
Lysyl hydroxylase
Lysyl oxidase
macropain subunit zeta
Macrophage inflammatory protein-1 alpha
Macrophage inflammatory protein-1 beta
Macrophage inflammatory protein-2 alpha
Macrophage inflammatory protein-3 alpha
Macrophage-stimulating protein (MST1)
Macrostatin
MAD-related protein 2
Major acute phase protein alpha-1
Major basic protein
Malic enzyme

Mannose receptor
MAP kinase kinase
Matrix metalloproteinase-1
Matrix metalloproteinase-2
MDM-2
MET proto-oncogene
Metallothionein 1
Metallothionein 2
Metal-regulatory transcription factor-1
Metastasis-associated mta1
Methionine adenosytransferase (MAT2A)
MHC class I
MHC class II
MHC class II transactivator
Mim
Mitochondrial ATP Synthase Subunit E
mitochondrial short-chain enoyl-CoA hydratase
Mitochondrial transcription factor 1
Mitogen activated protein kinase (P38)
Mitogen inducible gene (mig-2)
MOAT-B (MRP/organic anion transporter)
Monoamine oxidase A
Monoamine oxidase B
Monocyte chemotactic protein-1
Monocyte chemotactic protein-1 receptor (CCR2)
Mr 110,000 antigen
MSH3 gene
mss4, Zn2+ binding protein/guanine nucleotide exchange factor
Multidrug resistance-associated protein
Multidrug resistant protein-1
Multidrug resistant protein-2
Multidrug resistant protein-3 = cMOAT2
MUTL homologue (MLH1)
MutS Homologue (MSH2)
Myelin basic protein
Myeloid cell differentiation protein-1
Myeloid cell leukemia-1 (MCL-1)
Myeloperoxidase
Na/taurocholate cotransporting polypeptide
NADPH cytochrome P450 reductase
NADPH quinone oxidoreductase-1 (DT-Diaphorase)
Natural killer cell-enhancing factor B
N-cadherin
Neural cell adhesion molecule (N-CAM)
Neurofibromin (NF1 tumor suppressor)

neuropathy target esterase
NF-E2
NF-kappaB (p65)
Nidogen
Nitric oxide synthase-1, inducible
NMB
Non-specific cross-reacting antigen
Notch 1
Nucleic acid binding protein
Nucleoside diphosphate kinase beta isoform
nucleosome assembly protein
O-6-alkylguanine-DNA-alkyltransferase
OB-cadherin 1
Octamer binding protein 1
Octamer binding protein 2
Oncostatin M
Organic anion transporter 1
Organic anion transporter 3
Organic anion transporter K1
Organic anion transporting polypeptide 1
Organic cation transporter 1
Organic cation transporter 2
Organic cation transporter N1
Organic cation transporter N2
Ornithine decarboxylase
Osteocalcin
Osteopontin
Osteoprotegerin (TRAIL/Apo2L receptor)
OTK27
OX40 ligand
Oxygen regulated protein 150
Oxysterol-binding protein (OSBP)
Oxytocin receptor
p190-B (p190-B)
P311 HUM (3.1)
p53
p55CDC
p70 ribosomal protein S6 kinase alpha-1
Pancreatitis-associated protein
PAPS synthetase
PBX2 mRNA
P-cadherin
PCDH7 (BH-Pcdh)c
PDGF associated protein
PEG3
Perlecan
Peroxisomal 3-ketoacyl-CoA thiolase 1

Peroxisomal 3-ketoacyl-CoA thiolase 2
Peroxisomal enoyl-CoA hydratase: 3-hydroxyacyl-CoA dehydrogenase
Peroxisomal fatty acyl-CoA oxidase
Peroxisome assembly factor 1
Peroxisome assembly factor 2
Peroxisome biogenesis disorder protein-1
Peroxisome biogenesis disorder protein-11
Peroxisome biogenesis disorder protein-4
Peroxisome hydratase
Peroxisome proliferator activated receptor alpha
Peroxisome proliferator activated receptor gamma
Phenol sulfotransferase
Phenylalanine hydroxylase
Phosphatase 2A B56-alpha (PP2A)
Phosphoenolpyruvate carboxykinase
Phosphoglyceride kinase
Phospholipase A2
Phosphomannomutase (PMM2)
Pim1 proto-oncogene
Plasma cell membrane glycoprotein
plasma gelsolin
Plasminogen activator inhibitor 2
Platelet derived growth factor B
Platelet/endothelial cell adhesion molecule-1
Poly(ADP-ribose) polymerase
polyA binding protein
Presenilin-1
Prion protein (PrP)
pro-cathepsin L (major excreted protein MEP)
Progesterone receptor
Prohibitin
Prolidase
Proliferating cell nuclear antigen gene
Proliferation-associated gene A (natural killer-enhancing factor A)
prolyl 4-hydoxylase beta subunit (EC 1.14.11.2) (procollagen-L-proline, 2-oxoglutarate:oxygen oxidoreductase, 4-hydroxylating)
Prostacyclin-stimulating factor (IGFBP-7)
Prostaglandin H synthase
Prostate-specific antigen
protein disulfide isomerase
Protein kinase C alpha
Protein tyrosine phosphatase alpha
Protein-tyrosine phosphatase

Psoriasin 1 (S100 calcium-binding protein A7)
PTEN/MMAC1
Putative cyclin G1 interacting protein
Quinone reductase (zeta-crystallin)
RAD
RAD 51 homologue
RANTES
RAP1A (ras-related protein)
Recombination activating gene 1 (RAG-1)
Ref-1
RelB
Replication factor C, 40-kDa subunit (A1)
Replication protein A (70 kDa subunit)
Retinoblastoma
Retinoblastoma related protein (P107)
Retinoic acid receptor beta
Retinoic acid receptor gamma-1
Retinoid X receptor alpha
Retinoid X receptor beta
Retinoid X receptor gamma
Ribonucleotide reductase M1 subunit
Ribosomal protein L13A
Ribosomal protein L34 (RPL34)
Ribosomal protein L37a (RPL37A)
ribosomal protein S12
Ribosomal protein S4 (RPS4X) isoform
Ribosomal protein S9
RNA-dependent helicase
SAA-3
S-adenosylmethionine synthetase
Sarcoplasmic reticulum calcium ATPase
Sarcosin
Sec23B isoform, 2450bp
Senescence marker protein-30
Serine kinase
Serum amyloid A1
Serum amyloid A2-alpha
Serum response factor
Silencer of death domains (SODD)
Small proline-rich protein (sprl)
SMT3A protein
SMT3B protein
snRNP polypeptide B
Sodium/bile acid cotransporter
Sonic hedgehog gene
Sorbitol Dehydrogenase
SoxS

SPARC (secreted protein acidic and rich in cysteine)
Spermidine/spermine N1-acetyltransferase (SSAT)
Sphingomyelinase (neutral)
STAT 1
STAT 2
STAT 3
Stem cell factor
Steroid hormone receptor Ner-I
Sterol carrier protein 2
Sterol regulatory element binding protein-2
Stromelysin-1
Superoxide Dismutase Cu/Zn
Superoxide dismutase Mn
Supressor of cytokine signaling 1 (SOCS-1)
Supressor of cytokine signaling 3 (SOCS-3)
Survivin
Synapsin I
Synaptophysin II
Synaptotagmin I
Syntaxin 3
Tau protein
T-cell activation gene 3
T-cell cyclophilin
T-cell mRNA for glycyl tRNA synthetase
T-cell receptor
Tenascin
Thiol-specific antioxidant protein mRNA
Thiopurine methyltransferase
Thioredoxin
Thrombin receptor (PAR-1)
Thrombomodulin
Thrombospondin 2
Thymidine kinase
Thymidylate synthase
Thymosin beta-10
Tight junction protein Zo-1
Tissue factor
Tissue factor pathway inhibitor
Tissue inhibitor of metalloproteinases-1
Tissue inhibitor of metalloproteinases-3
Tissue transglutaminase
TNF receptor-1 associated protein (TRADD)
transcription elongation factor S-II, hS-II-T1
Transcription factor IID
transcriptional activator hSNF2b

Transferrin
Transferrin receptor
Transforming growth factor-beta 3
Transthyretin
Tropoelastin
Tryptophan hydroxylase
Tryptophanyl-tRNA synthetase
ts11 gene encoding a G-1 progression protein
Tumor necrosis factor associated factor 2 (TRAF2)
Tumor necrosis factor receptor 1
Tumor necrosis factor receptor 2
Tumor necrosis factor receptor-1 associated protein (TRADD)
Tumor necrosis factor-alpha
Tumor necrosis factor-beta
Type 1 interstitial collagenase
Tyrosine aminotransferase
Tyrosine hydroxylase
Tyrosine protein kinase receptor (UFO)
U1 small nuclear RNP-specific C protein
Ubiquitin
Ubiquitin conjugating enzyme (Rad 6 homologue)
Ubiquitin conjugating enzyme G2 (UBE2G2)
Ubiquitin-homology domain protein PIC1
UDP-glucuronosyltransferase 2
UDP-glucuronosyltransferase 2B
Uncoupling protein 1
Uncoupling protein 2
Uncoupling protien 3
Urate oxidase
Urokinase plasminogen activator receptor (uPAR)
UV excision repair protein RAD 23 (XP-C)
Vascular cell adhesion molecule 1 (VCAM-1)
Vascular endothelial growth factor
Vascular endothelial growth factor D
Vascular endothelial growth factor receptor 1 (flt-1)
Very long-chain acyl-CoA dehydrogenase
Vesicle-associated membrane protein-2 (VAMP-2)
Vesicular acetylcholine transporter (VAChT)
Vesicular monoamine transporter (VMAT)
Vimentin
Visinin-like peptide 1 homolog
Vitellogenin

Waf1
Wnt-13 mRNA
X13694
Zinc finger protein ZNF134
Zinc finger protein
Zinc-finger DNA-binding motifs (IA-1)
Zinc-finger protein-37
Zipper protein kinase (ZPK)
Serum paraoxonase

5

TABLE 5

Renal Toxicity	Neural Toxicity
Alpha-2 microglobulin	Acid ceramidase
Bile salt export pump (sister of p-glycoprotein)	Ataxia telangeictasia
Calbindin-D (28kDa)	Brain-derived neurotrophic factor
Calbindin-D (9K)	Brain-derived neurotrophic factor
Calcineurin-B	Choline kinase
Calnexin	Cystic fibrosis transmembrane conductance regulator
Cholesterol esterase	Dopamine beta-hydroxylase
endothelin-1	Dopamine receptor D2
FGF-1	Dopamine transporter
FGF-7	Endothelin-1
Gamma glutamylcysteinyl synthetase	Glial fibrillary acidic protein
Gamma-glutamyl hydrolase precursor	Glutamine synthetase
Gamma-glutamyl transpeptidase	Myelin basic protein
Heat shock protein 90	Nerve growth factor
Kidney injury molecule-1	Nerve growth factor receptor
NMB	Neural cell adhesion molecule
Organic anion transporter 1	Neuropathy target esterase
Organic cation transporter 1	Synapsin I
p-glycoprotein (MDR-1)	Synaptophysin
Phosphoenolpyruvate carboxykinase	Synaptotagmin I
Sphingomyelinase, neutral	Tau protein
Vimetin	Vesicular acetylcholine transporter
MOAT-B (MRP/organic anion transporter)	Vesicular monoamine transporter
Organic anion transporter 1	Norepinephrine transporter
Organic anion transporter 3	Serotonin N-acetyltransferase
Organic anion transporter K1	Serotonin transporter (SERT)
Organic anion transporting polypeptide 1	Sphingomyelinase (neutral)
Organic cation transporter 1	
Organic cation transporter 2	
Organic cation transporter 3	
Osteopontin	
Renal organic anion transporter	

Hepatic Toxicity	Immunotoxicity
11-beta hydroxysteroid dehydrogenase type II	
12-lipoxygenase	6-C-kine
15-hydroxyprostaglandin dehydrogenase	Complement component C3
17-beta hydroxysteroid dehydrogenase	Cyclooxygenase 2
25-hydroxyvitamin D3-1 alpha-hydroxylase	Eosinophil-derived neurotoxin
Alanine aminotransferase	Eotaxin
Alcohol dehydrogenase 1	Granulocyte-macrophage colony-stimulating factor
All Cytochrome P450 genes	IkB-a
Alpha 1-antitrypsin	Interferon gamma
Bile salt export pump (sister of p-glycoprotein)	Interferon inducible protein 10
Bilirubin UDP-glucuronosyltransferase isozyme 1	Interferon inducible protein 15
Biliverdin reductase	Interferon stimulatory gene factor-3
Branched chain acyl-CoA oxidase	Interleukin-1 alpha
Canalicular multispecific organic anion transporter	Interleukin-1 beta
Carnitine palmitoyl-CoA transferase	Interleukin-10
Catechol-O-methyltransferase	Interleukin-12
Cholesterol esterase	Interleukin-13
Corticosteroid binding globulin	Interleukin-18
Enoyl CoA hydratase	Interleukin-2
Epoxide hydrolase	Interleukin-8
Fatty acid synthase	Interleukin-4
Fatty acyl-CoA oxidase	Interleukin-5
Fatty acyl-CoA synthase	Interleukin-6
Flavin containing monooxygenase 1	Macrophage inflammatory protein-1 alpha
Focal adhesion kinase (pp125FAK)	Macrophage inflammatory protein-1 beta
Gamma glutamylcysteinyl synthetase	Macrophage inflammatory protein-2 alpha
Gamma-glutamyl hydrolase precursor	Macrophage inflammatory protein-2 beta
Gamma-glutamyl transpeptidase	Macrophage inflammatory protein-3 alpha
Glucose-regulated protein 58	Macrophage inflammatory protein-3 beta
Glutamic-oxaloacetic transaminase	Macrophage metalloelastase
Glutamic-pyruvic transaminase	MHC class 1
Glutathione S-transferase Ya	MHC class 2
Hepatic lipase	MHC class 2 transactivator
Hepatocyte growth factor	Monocyte chemotactic protein receptor (CCR2)
Hepatocyte growth factor receptor	Monocyte chemotactic protein-1
Hydroxysteroid sulfotransferase a	Neutrophil elastase
Na/taurocholate cotransporting polypeptide	Phospholipase A2

Senescence-marker protein-30
Hepatocyte growth factor activator
Lipopolysaccharide binding protein
Liver fatty acid binding protein
Major acute phase protein alpha-1
NADPH cytochrome P450 reductase
Peroxisomal 3-ketoacyl-CoA thiolase 1
Peroxisomal 3-ketoacyl-CoA thiolase 2
Peroxisomal acyl-CoA oxidase
Peroxisomal enoyl-CoA hydratase: 3-hydroxyacyl-CoA dehydrogenase
Peroxisomal fatty acyl-CoA oxidase
Peroxisome assembly factor 1
Peroxisome assembly factor 2
Peroxisome biogenesis disorder protein-1
Peroxisome biogenesis disorder protein-11
Peroxisome biogenesis disorder protein-4
Peroxisome hydratase
Peroxisome proliferator activated receptor alpha
Peroxisome proliferator activated receptor gamma
Serum amyloid A1
Serum amyloid A2-alpha
Transthyretin
Suppressor of cytokine signaling 1
Suppressor of cytokine signaling 3
T-cell activation gene 3
T-cell cyclophilin

Cardiotoxicity	Pulmonary Toxicity
Adrenomedullin	GARG-16
Atrial natiuretic factor	GAS-7
Endothelin-1	IRF-7
Glucose transporter 1	ISG-15
Nitric oxide synthase-1, inducible	Lipocalin
Osteopontin	Liposin
Protein kinase C - beta 1	Macrostatin
RhoA	MME
Sarcoplasmic reticulum calcium ATPase	MRP14
Vascular endothelial growth factor	MRP-8
	Osteopontin
	SAA-1
	SAA-3
	Tenascin
	Tropoelastin

TABLE 6

Apoptosis	Cell Cycle
Adenine nucleotide translocator 1	Activating transcription factor 2
Annexin V	Ataxia telangeictasia
BAK	c-myc
Bax (alpha)	Cell division cycle protein 2
Bcl-xL	Cell division cycle protein 20
c-myc	Cell division cycle protein 25
Calcineurin-B	Checkpoint kinase-1
Calprotectin	Cyclin D1
Caspase 1	Cyclin dependent kinase 1
Caspase 2	Cyclin dependent kinase 4
Caspase 3	Cyclin dependent kinase inhibitor 1A
Caspase 4	Cyclin E
Caspase 6	Cyclin G
Caspase 7	Cyclin-dependent kinase 4 inhibitor B (P15)
Caspase 8	Cyclin-dependent kinase 4 inhibitor B (P16)
Clusterin	Cyclin-dependent kinase 4 inhibitor P27kip1
Cyclin dependent kinase inhibitor 1A	Dihydrofolate reductase
Cyclin-dependent kinase 4 inhibitor P27kip1	DNA binding protein inhibitor ID2
Dynein light chain 1	E2F-1
E2F-1	GOS24 (zinc finger transcriptional regulator)
Fas antigen	MDM-2
Fas associated death domain (FADD)	p53
Fas ligand	p55CDC
Gadd153	Retinoblastoma
Interleukin-12	T-cell cyclophilin
p53	Transcription factor IID
Retinoblastoma	Ubiquitin-homology domain protein PIC1
Thymosin beta-10	Waf1
Tumor necrosis factor receptor 1	
Tumor necrosis factor receptor-1 associated protein (TRADD)	
Waf1	

Cell Proliferation	DNA Damage
Activin beta E	Activating transcription factor 2
Activin receptor type II	Ataxia telangeictasia
c-abl	ATP-dependent helicase II (70kDa)
c-erb A-1	ATP-dependent helicase II (Ku80)
c-fos	BRCA1
c-jun	c-abl
c-myc	Cell division cycle protein 20
Early growth regulated protein 1	Checkpoint kinase-1
Endothelin-1	Cyclin D1
Extracellular-signal-regulated kinase 1	Cyclin-dependent kinase 4 inhibitor B (P16)
FosB	DNA dependent protein kinase
GOS24 (zinc finger transcriptional regulator)	DNA ligase I
GT mismatch binding protein	DNA ligase IV
Hepatocyte growth factor receptor	DNA polymerase beta
ID-1	DNA repair and recombination homologue (RAD 52)
Insulin-like growth factor II	DNA repair protein (RAD 50)
Interleukin-6	DNA topoisomerase I
L-myc	DNA topoisomerase II
MutS homologue (MSH2)	Dynein light chain 1
Proliferating cell nuclear antigen gene	ERCC 1 (excision repair protein)
Replication protein A (70 kDa subunit)	ERCC 3 (DNA repair helicase II)
Ribosomal protein L13A	ERCC 5 (excision repair protein)
Thrombospondin 2	ERCC 6 (excision repair protein)
Thymidine kinase	FEN-1 (endonuclease)
Thymidylate synthase	Gadd153
Transforming growth factor-beta3	Gadd45
	GT mismatch binding protein
	JNK1 stress activated protein kinase
	L-myc
	MDM-2
	MutS homologue (MSH2)
	Nucleoside diphosphate kinase beta isoform
	O-6-alkylguanine-DNA-alkyltransferase
	p53
	p55CDC
	Poly(ADP-ribose) polymerase
	Proliferating cell nuclear antigen gene
	RAD 51 homologue
	Ref-1

	Replication protein A (70 kDa subunit) Retinoblastoma Transcription factor IID Ubiquitin conjugating enzyme (RAD 6 homologue) UV excision repair protein RAD 23 (XP-C) Waf1

Inflammation	Peroxisome Proliferation
12-lipoxygenase	17-beta hydroxysteroid dehydrogenase
Apolipoprotein AII	Apolipoprotein CIII
C-reactive protein	Bilirubin UDP-glucuronosyltransferase isozyme 1
Calprotectin	Branched chain acyl-CoA oxidase
Cyclooxygenase 2	Carnitine palmitoyl-CoA transferase
Fas ligand	Cytochrome P450 4A
IkB-a	Cytochrome P450 4A1
Intercellular adhesion molecule-1	Enoyl CoA hydratase
Interleukin-1 alpha	Epoxide hydrolase
JNK1 stress activated protein kinase	Farnesol receptor
NF-kappaB (p65)	Fatty acyl-CoA oxidase
Nitric oxide synthase-1, inducible	Glucose-regulated protein 58
Phospholipase A2	GOS24 (zinc finger transcriptional regulator)
Serum amyloid A1	Hepatic lipase
Serum amyloid A2-alpha	Lipoprotein lipase
Tumor necrosis factor associated factor 2 (TRAF2)	Liver fatty acid binding protein
Tumor necrosis factor receptor 1	Malic enzyme
Tumor necrosis factor receptor 2	Peroxisomal 3-ketoacyl-CoA thiolase 1
	Peroxisomal acyl-CoA oxidase
	Peroxisomal enoyl-CoA hydratase: 3-hydroxyacyl-CoA dehydrogenase
	Peroxisome assembly factor 1
	Peroxisome assembly factor 2
	Peroxisome biogenesis disorder protein-1
	Peroxisome proliferator activated receptor alpha
	Peroxisome proliferator activated receptor gamma
	Retinoid X receptor alpha
	Uncoupling protein 1
	Uncoupling protein 2
	Uncoupling protien 3
	Urate oxidase
	Very long-chain acyl-CoA dehydrogenase

TABLE 7

1-chloro-2-nitrobenzene
2,4-dinitrophenol
2-acetylaminofluorene
2-azido-2-deoxycytidine
2-azido-2-deoxyuridine
4-acetamidofluorene
5-azacytidine
5-chlorouracil
5-fluorouracil
6-mercaptopurine
6-thioguanine
acetamidofluorene
acetaminophen
acetylsalicylic acid
acridine
actinomycin
allyl alcohol
aminopterin
aminotriazole
antimycin a
antipyrine
benz[a]pyrene
bleomycin
busulfan
caffeine
camptothecin
carbamazepine
carbon tetrachloride
carboplatin
carmustine
chlorambucil
chloroquine
cimetidine
cisplatin
clenbuterol
clofibrate
clozapine
colchicine
corticosterone
cycloheximide
cyclophosphamide
cyclosporin
cytosine arabinoside
dacarbazine
dt-5-fluorouracil
erythromycin
ethyl methanesulfonate
etoposide
fenofibrate
flufenamic acid
gemfibrozil
guanine
hdp527
hydroxyurea
icrf
icrf/doxorubicin
indomethacin
iodoacetamide
isonicotinic acid
M077
mechlorethamine
melatonin
melphalan
methotrexate
methyl methanesulfonate
mitomycin c
mitoxantrone
n-nitroso-n-ethylurea
n-nitroso-n-methylurea
naloxone
naproxen
nicotine
nitrofurantoin
o-toluidine
oligomycin
paclitaxel
PGU693
phenobarbital
phorbol 12-myristate 13-acetate diester
prednisone
proflavin
progesterone
puromycin
rezulin
rifampicin
rosiglitazone
sodium azide
streptozotocin

dexamethasone
diethylhexylpthalate
diethylstilbestrol
diflunisol
digitoxin
dimethylhydrazine
dmso
doxorubicin
tacrine
tamoxifen
thioguanine
transplatin
triethylenemelamine
triethylenethiophosphoramide
verapamil
wy 14,643

TABLE 8

1	Activating transcription factor 4
2	Activin receptor type II
3	Ataxia telangeictasia
4	c-H-ras
5	c-jun
6	Carnitine palmitoyl-CoA transferase
7	complement component C3
8	Cytochrome P450 1A1
9	DNA dependent helicase
10	DNA mismatch repair protein (PMS2)
11	Epoxide hydrolase
12	ERCC 5 (excision repair protein)
13	ERCC 6 (excision repair protein)
14	Farnesol receptor
15	Gadd45
16	Glucose-6-phosphate dehydrogenase
17	Glutathione peroxidase
18	Histone 2B
19	Interleukin-1 alpha
20	Interleukin-6

TABLE 9

CELL TYPES IN THE HEART:
Myocytes (cardiac muscle cells)
Vascular endothelial cells
Purkinje cells - regulate rate and rhythm of the heart
CELL TYPES IN THE LUNG:
Columnar, ciliated epithelial cells - line trachea, bronchi, bronchioles
Goblet cells - secrete mucus
Neuroendocrine cells - contain serotonin, calcitonin and gastrin-releasing peptide
Capillary endothelial cells
Interstitial fibroblast cells
Smooth muscle cells
Mast cells - in pulmonary interstitium
Type I alveolar (epithelial) cells - compose 90% of alveolar surface
Type II alveolar (epithelial) cells - secrete surfactant and mediate repair of alveolar epithelium
Alveolar macrophages
Serous cells - produce a fluid to dissolve mucus
Brush cells (Type III epithelial cells)
Clara cells - highly metabolic
Parenchymal cells (connectivity tissue cells)
CELL TYPES IN THE KIDNEY:
Capillary endothelial cells
Visceral epithelial cells (podocytes) - form glomerualr barrier
Parietal epithelial cells - line Bowman's space
Mesangial cells - have contractile abilities to reduce amount o glomerular surface available for filtration
Tubular epithelial cells
Juxtaglomerular cells (modified granulated smooth muscle cells; also called granular cells)
Lacis cells (non-granular cells)
Fibroblast-like cells
Macula densa cells (specialized tubular epithelial cells)
CELL TYPES IN THE BRAIN:
Neurons
Astrocytes - found in gray and white matter; responsible for repair and scar formation
Oligodendrocytes - main component of white matter; produce and maintain CNS myelin
Microglia cells - serve as macrophage-type cells
Ependyma cells (columnar epithelial-like cells with a ciliated border) - line the ventricular system
Fibroblasts
Capillary endothelial cells
Meningeal fibroblast cells
Leptomeningeal (mesenchymal) cells
Purkinje cells
Meningothelial cells

Macrophages
CELL TYPES IN THE LIVER: Kupfer cells - resident macrophages Sinusoidal endothelial cells Ito cells (lipocytes) - synthesize collagen and store vitamin A Hepatocytes (parenchymal cells) - majority of cells in the liver are of this type Bile duct epithelial cells Hepatic venule endothelial cells Sinusoidal epithelial cells

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
1	A + AP8	Inhibitor of apoptosis protein-1	310bp	AAGCTTTTACCGCTGAGAATGATGA GGATGAGAATGGTGGTTGAAGGTT ACATTTTAGGAAATGAGGAAACTTA GAAAATTAATATAAAGACAGTGATG AATACAAAGAAGATTTTTATAACAA TGTGTAAAATTTTTGGCCAGGGAAA GGAATATTGAAGTTAGATACAATTA CTTACCTTTGAGGGAAATAATTGTT GGTAATGAGATGTGATGTTTCTCCT GCCACCTGGAAACAAAGCATTGAA GTCTGCAGTTGAAAAGCCCAACGT CTGTGAGATCCAGGAAACCATGCT TGCAAACCACTGGT	Repressed in Individual 1 and constitutive in Individual 2	AF070674
2	A + AP7	No significant match to anything	200bp	AAGCTTAACGAGGAAAATATATCAG TTAAATATTTAGGTTGACATTGTTA GGAACCAAGGTTTTTAACAAATGAN AAAAAAAGCCGGGTGCGGTGGTTC GTGCCTGTAATCCCAACACTTTGG AAGGCTGAGGTGGGAGGATCACTT GAGCTCAGGAGTTTGAGACCAACC TGGGCAACATAGCCAGACCTCACT CTACT	Repressed in Individual 1 and constitutive in Individual 2	
3	A + AP5	Gu protein (nucleolar RNA helicase recognized by autoimmune antibodies from a patient with watermelon stomach disease)	124bp	ACATACTCTTGAGCAATGCTAATCT GCGCCCCTTACTCCCTTAAGTCCT TCTTGGTAAATAATGTTAATCTTCC AATAGGAAGAAGTGGAGTACATTA CCATTTAAGCACCATTTATCCAGCC TACTAAGCTT	Repressed in Individual 1 and constitutive in Individual 2	AF261917

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
4	A + AP7	Ribosomal protein L34	117bp (65bp match)	GGAAGCTTAACGAGGAGCAGAAAA TCGTTGTGAAAGTGTTGAAGGCAC AAGCACAGAGTCAGAAAGCTAAAT AAAAAAATGAAACTTTTTTGAGTAA TAAAAATGAAAAGACGCTGT	Repressed in Individual 1 and constitutive in Individual 2	NM_000995
5	G + AP5	clone 459L4 on chromosome 6p22.3-24.1	171bp	AAGCTTAGTAGGCAAATAGGTTTGT TGATGATGAAGTGGTTTGTGTTTTA TAAGAGTCCTTATGGTCTAGATGG CATTTGAACTTGTGGTTCCAGTGAT TACCAGGACCCTGAATTGAAGAAT ACATGCTGTGGCCTTTTCAGCTCA GGAAACTTTTTACTTTCTATTGCC	Repressed in Individual 1 and constitutive in Individual 2	AL031120
6	C + AP6	eukaryotic translation initiation factor 3 (subunit 10 -theta)	126bp (86bp match)	AAGCTTGCACCATATCCACAGGAG GTTGGAAAAACCATGCCATTTTCTG GAATTTAAGGGTGTTGCATTATTTC ATCAATCATTTGTTGACAAAAAAGA AAAACTAAAAAATAAATTTAAAATGT G	Repressed in Individual 1 and constitutive in Individual 2	NM_003750
7	G + AP7	ribosomal protein S9	94bp (86 bp match)	GTAAAGCGCTGATCCTGTTTATTTG GCAGGAAAACGAGACAATCCAGCA GCCCAGGAGGGACAGGTGGACTT AATCCTCCTCCTCGTTAAGCTT	Induced in Individual 1 and Individual 2	NM_001013
8	C + AP6	No significant match to anything	182bp	AAGCTTGCACCATGAACAAAGGAA TCATCCCTAATATGTGATTAACTGA CGTTCCCTGCTCACCACAAATGTT GGCTTTCATAGACTTCAAGTGAAG GGGCACTTTATTAATTGGCTGAAGA ATTCCTTAGAACGCCTAAGAGATTA AGACCACAGGATGCTTCAAGGTTC TTTCTTTCTTG	Repressed in Individual 1 and constitutive in Individual 2	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
9	C + AP7	No significant match to anything	98bp	CCAAGATGCCGGGCACTGCACGG CTTTTATCAGTTTTTATTACATCAG GTCAAGGAAACAAATGGAATCTATC CACATGGTCCTGCCTCGTTAAGCT	Absent in Individual 1 and repressed in Individual 2	
10	G + AP6	KIAA1225 protein	252bp	AAGCTTAGTAGGCAGCACTTTTAAA AATATGTGAACTCAAATATTGCACT TCTTTCAAGATGTTATCAATTGGTT ATTGTACTGTATAGTTTTAATAATTT TGATTGAAACCCTTTAACAACTCTT TGTAAATTTTAACTCATTTTAGTTGA TTTTCAGTACTATTTACATAGGAATT GATTTTTATGGATATAGTAGAAGAA ATGTGCTGTATTTTGATAAAATTCA CTTATTGTATGTGTGTTGTAATCT	Constitutive in Individual 1 and repressed in Individual 2	AB033051
11	A + AP5	erbb2-interacting protein ERBIN (KIAA1225 protein)	252bp	AGATTACAACACACATACAATAAGT GAATTTTATCAAAATACAGCACATT TCTTCTACTATATCCATAAAAATCAA TTCCTATGTAAATAGTACTGAAAAT CAACTAAAATGAGTTAAAATTTACA AAGAGTTGTTAAAGGGTTTCAATCA AAATTATTAAAACTATACAGTACAAT AACCAATTGATAACATCTTGAAAGA AGTGCAATATTTGAGTTCACATATT TTTAAAAGTGCTGCCTACTAAGCTT	Repressed in Individual 1 and constitutive in Individual 2	NM_018695
12	G + AP7	angiotensin II receptor (AGTR1)	76bp	AAGCTTAACGAGGCTATCAGCAAA CAAAGAGAAGAGGCCCAGGTGGG CTCAATAGCCAGGTCACAAATGGT TGCCC	Repressed in Individual 1 and induced in Individual 2	AF245699
13						

Band	Primer Combination	Identification	Size	Sequence	Expression In Individuals Tested	GenBank Accession number
14	C + AP11	Alpha-enolase	153bp	AAGCTTCGGGTAATTGGCCCAAGT CATTGTTTTTCTCGCCTCACTTTCC ACCAAGTGTCTAGAGTCATGTGAG CCTCGTGTCATCTCCGGGGTGGCC ACAGGCTAGATCCCCGGTGGTTTT GTGCTCAAAATAAAAAGCCTCAGT GACCCATG	Constitutively high in Individual 2 and induced in Individual 1	AF035286
14	C + AP11	Calmodulin 3	152bp	CAGGTAGTCACTGTATTTTATTGGA AAACATTGATATATATTTTTCTTCAC AGCTTGAACTGAACACAATATTGCC CGGTTTAAAAAAAACAAAACCAAAA CATTCCGAAAATGTCCACAGCCTC ACGCCTACCTGCCCTTACCCGAAG CTT	Constitutively high in Individual 2 and induced in Individual 1	NM_005184
15						
16						
17						
18	A + AP19	No significant match to anything	91bp	AAGCTTATCGCTCCCACTCCAGAG AAACTTTAATGCTCAGGCTTCAAAC TCCCTATCTTCCCTCCTCAGAGGT CCTTCTGTCCCCTTTACT	Repressed in Individual 2, but not in Individual 1	
19						
20	A + AP19	Clone RG013F03	126bp (93 match)	AAGCTTATCGCTCGCAGGGTGTTC CGTAGTTTCTTCTCGAGCCAATGCA TGTATTATAGCAGCAGGTGTCTTTG TGCTTTCTCATCATAGTAACGTACT ACTTGTAAATACATTTTTCTATTTTC	Absent in Individual 2 and induced in Individual 1	AC005046

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
21	A +AP23	clone 1189B24 on chromosome Xq25-26.3.	289bp	ACTCTTGAAGATCTGTTAACTATGA TGATAAAATTATATTTCCTCCCAAT CAAGAAATATTATACATGACTTTAA AAAAACTATCATCCAGTATAGACCG GAAGTTGATAATCAGAAGTGGTAA CTCTCACCAGCCTGTGGAAACATG ATCGCATAATCTCCCATAATCTCCT GAAGGCAGAAGATAAACTTCAACA CTGTAAAACATTTTAAAGTCTTCAG AATCCTTCTGTCATCTAGTCTTGTA AATCACATTAAAATATAATTTTGTTA CCCCATAGCCAAGCTT	Repressed in Individual 1 and constitutive in Individual 2	AL030996
22	C + AP22	Interferon, gamma-inducible protein 16	135 (71 match)	AAGCTTTTGATCCTATGGAATGGG GTATTGGGAGTGCTTTTTTAATTTT TCATAGTTTTTTTTTAATAAAATGGC ATATTTTGCATCTACAACTTCTATAA TTTGAAAAAATAAATAAACATTATCT TTTTTGTG	Constitutive in Individual 2 and repressed in Individual 1	AF208043
23	C+AP17	Kinectin 1 (kinesin receptor)	183bp	AAAGCTTACCAGGTGTTAGAGTGA AGTAATTGGGAAACTGTTCATTTGA GGATAAAAAAGGCATTGTATTATAT TTTGCCAAATTAAAGCCTTATTTAT GTTTTCACCCTTTCTACTTTGTCAG AAACACTGAACAGAGTTTTGTCTTT TCTAATCCTTGTTAGACTACTGATT TAAAGAAGG	Constitutive in Individual 1 and repressed in Individual 2	NM_004986
24						
25	C + AP 24	KIAA0911 protein	119bp	CCAAACTAGTGCATGTATAAATAAT GGCAGGATGGGGGGTACTGTGTA GATGATTAACTGACTTTTTAATATTT TGTAAATAAATCGGATTCCTTGTGT CCTTTGTGCTAGTGAAGCTT	Constitutive in Individual 2 and induced in Individual 1	NM_014944

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
26	G + AP28	No insert - just vector sequence				
27						
28						
29						
30						
31	C + AP26	No insert - just vector sequence				
32	A + AP28	Ribosomal protein S12	80bp	ACATCACAAGCGCCAGGGTCTGTT TATTAAAACCCCTTCCCAGCAGAG GGCAGTACAGTGTCTTGTCCCAGC AGAAGCTT	Repressed in Individual 2 and constitutive in Individual 1	AF058761
33	C + AP27	KIAA0890 protein	69bp	CACAAGGGATAAATAGAACTTTATT TTAAATAAACATTTGCACTCTGTAC ACAGCCCCAGCAGAAGCTT	Induced in Individual 3, but not in Individual 4	NM_014966
34	C + AP29	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit e (ATP5I)	149bp	CACACAACACAGGAAGCTTTATTCA TCCGCTGCTGGTCCAAAGAGTGGG TCGCAGGGTCACTCACTTTAATATG CTGTCATCTTCTGCCAATTCTCTGG CAATCCGTTTCAGTTCATCCTGCTT CTTCTTCTCTTCTGCTGCTAAGCTT	Repressed in Individual 3, but not in Individual 4	NM_007100
35	G + AP31	carbonyl reductase	175bp	GGGCAGCAGAATCAGGTTTATTGG AGGGATTGGGGGTAGGATGAGCAC GGCATGGGGCTTGAGGTGTGTGG AGGGAGCTCAGCAGGCCCAGAAG CCCCCTTCCACCGGCAAAGTGGAA CCCGTGGTCATGCCACTTCGGTCA CTCAGCAGAAAGAGGATGGCGTTC ACCAAGCTT	Slightly induced in Individual 4, but not in Individual 3	NM_016286
36						

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
37	A + AP10	KIAA1224 protein	223bp	ACAGGTTTCATCTGAATACATATTT ATTAGATAAATATTAGAGGTTGTCA CATCATCTAACTACATACAGCTTTG CAAGACTAGAAATCACAATTAGTTT TTTGACCAGTTTAAAGTATGAAATG ATTGCATTGTACATACGATGTACAA AGACGATGATGGTTTCTGTGGGAG TTACTTCAGGCTGCACTGGTGGGT GTGTTTATGTGTGTACGTGGAAGC	Reduced in Individual 4 and constitutive in Individual 3	AB033050
38	G + AP11	CAMPATH-1 (CDw52) antigen	71bp	GCAAGTNCAAGGGTNACTTTATTG ACCCCCAGCTGGGCACTGNTNGNC CCCTACATCATTACCCGAAGCTT	Induced in Individual 4, constitutive in Individual 3	X62466
38	G + AP11	tumor necrosis factor type 2 receptor associated protein (TRAP3)	71bp	GANATTTGAATGGTTTAATAACTCT GTCTTCGTGAGCTGGATGGCAAGC TCTCACACTTTACCCGAAGCTT	Induced in Individual 4, constitutive in Individual 3	NM_021138
39						
40	G + AP10	Insert size doesn't match the autorad	54bp	AAGCTTCCACGTAACCACAGCCCT AAATCCATGATAAAGCTTCCACGTA CTACC	Repressed in Individual 4, constitutive in Individual 3	
41	C + AP9	Sperm autoantigenic protein 17 (SPA17)	216bp (126 match0	CACAACAGTAACGAATGTTATTTCA CAACATCAAATCTTCCTTCCTCAGG AAGAAATGACATTAATAAGAAGGTT GATGGATTTGGATTATTTTTCATGT TTCCTGGAGGTAAAACCAGTGTCC TCACTTGTTTTCCTCTTTTTCCTCAT TTTGAAGACTATTTGTTTTCATTTTC TTTGCCTCCTCTCTGGCTATGTGTC CCCGGAATGAAGCTT	Constitutive in Individual 3 and repressed in Individual 4	NM_017425
42	C + AP10	No insert - just vector sequence				

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
43	C+AP16	A kinase anchor protein 2 (PRKA2)	30 bp (matches 19)	CAATTATTGCCGCTCTAAAGCTTCC TGAAT	Constitutive in Individual 4 and repressed in Individual 3	NM_007203
44	G + AP23	growth arrest specific transcript 5 gene	314bp	AAGCTTGGCTATGGAGAGTCGGCT TGACTACACTGTGTGGAGCAAGTT TTAAAGAAGCAAAGGACTCAGAATT CATGATTGAAGAAATGCAGGGTTTT TAATGACCACAACAAGCAAGCATG CAGCTTACTGCTTGAAAGGGTCTT GCCTCACCCAAGCTAGAGTGCAGT GGCCTTTGAAGCTTACTACAGCCT CAAACTTCTGGGCTCAAGTGATCC TCAGCCTCCCAGTGGTCTTTGTAG ACTGCCTGATGGAGTCTCATGGCA CAAGAAGATTAAAACAGTGTCTCCA ATTTTAATAAATTTTTGCAATCC	Induced in Individual 4, but not in Individual 3	AF141346
45	G + AP19	5-aminolevulinate synthase 2 (ALAS2)	255bp	AAGCTTATCGCTCAAAGTCTCCTAC CCTTCTCTTCCTCTTTTAAGCCCTC TCTTCCTCGCTTTCCTTCCTACCTA GCTCCTTGTTGGTGAGCTTCTTGT GCCTTAATCCTGTGACCCAGCCCC TTACACCACTTTCCACCTTCCTGTC CGAAGTACACGGACACTAGCTGCC CCAGGAAGTTGTGTGATTTTAAATC ACTTCTGTCTTTGCTGGAAAGTGTA TTTGTGCATAAATAAAGTCTGTGTA TTTGTTTC	Induced in Individual 4, but not in Individual 3	AF068624
46	G + AP23	Clone NH0523H20	101bp	GGGTGGAGCGGGTACTTTATGAAT ACCACAGGGACAAAGGAAGGCTGC TCTTNTCACACACTGCTGAATCTCC GGATCTCCGCAAGGTCATAGCCAA GCTT	Constitutive in Individual 3, increased in Individual 4	AC005041

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
47		Very tiny insert				
48	G + AP23	Low molecular mass ubiquinone-binding protein	131bp	GCANACCATAGGAAGTTTATTGTGT CTTTGANACACTACAATGCAGACTC CTCTTCCANAGAAAGGTCTTTCAAA GACAGGGAACCGTCATCCGGATGC GTTGCTCATTTGTCATTTTCATAGC CAAGCTT	Constitutive in Individual 3, increased in Individual 4	NM_014402
49	G + AP23	ATP synthase, H+ transporting, mitochondrial F1F0, subunit g (ATP5JG)	118bp	AAGCTTGGCTATGATGTTTGAAGAC CAATCTTTAACATCTGATTATATTTG ATTTATTATTTGAGTGTTGTTGGAC CATGTGTGATCAGACTGCTATCTGA ATAAAATAAGATTTGTC	Repressed in Individual 3, but not in Individual 4	NM_006476
50	C + AP24	phosphoglycerate kinase 1	173bp	AAGCTTCACTANCACAATGTCTGCC ATAAAGTAGGCCCTTGATAAAGAAT GGACATTTATCTAATTGTCCCATCT CTCCACTGCTGCTGCCCCACTTCT TGCATTCAGCAATATANACATCTGA TCCGTTCCTCAAGATTCTATTCTCA CCCTTCCTAACAAAGTATGACAGG	Induced in Individual 4, but not in Individual 3	S75476
51	A + AP23	Acid phasphatase 1	274bp	ATATCTCTAGTTGTATTTTTATAACT CCCCAGTTTATTTTGAAATATTCAT GATTTTGACATTATCTCAAAATACA CAGAAATTACCTTACATCTGCCTAT ACATTTATTAAATGCTGATGAAGAA TACTTATTCAATATTTAAGCATGCC AAATAATAACACTTTTCCCAACTTTT AAAATTATTTTAAAAAATAAGCCAAT TTATATTGGACATTTGGGTTACATG TGCATATAAAAACAGTCATATTCCT CAACTGACCATAGCCAAGCTT	Constitutive in Individual 3, repressed in Individual 4	NM_007099

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
52	C +AP20	Clathrin, heavy polypeptide-like 2 (CLTCL2)	246bp	CCACAGTCCAGTAATTTATTTTAAA TTTGAGTAATTTCAAATTCCACAAA CAAAACTGAAGAACAGCAATATTTT GTTTGAATTTCTCTCTTCTGTACAC TCAGTGATCTAAAACACCACAATAT CCAACATACACAAACCTCAGGGAA GGGTTAGTAAACACACACAAGATT GGAATCATGGTGCTCTTTGCTCCT GAATGGAATGGTCCCACAGAAAAA GCACAGGATACAGCACAACAAGCT T	Induced in Individual 3, repressed in Individual 4	NM_004859
53	C + AP21	MHC class II HLA-DR-beta (DR2-DQw1/DR4 DQw3)	223bp	AAGCTTTCTCTGGACCTGGTTGCTA CTGGTTCGGCAACTGCAGAAAATG TCCTCCCTGGTGGCTTCCTCAGCT CCTGCCCTTGGCCTGAAGTCCCAG CATTGATGGCAGCGCCTCATCTTC AACTTTNGTGCTCCCCTTTGCCTAA ACCGNATGGCCTCCCGTGCATNTG TATTCACCCTGTATGACAAACACAT TACATTATTAAATGTTTCTCAAAGAT GG	Repressed in Individual 3, but not in Individual 4	M20430
54	A + AP23	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex	250bp	AAGCTTGGCTATGGTTAAAGCGGA ACCAGATGTTAAAAAATTAGAAGAC CAACTTCAAGGCGGTCAATTAGAA GAGGTGATTCTTCAGGCTGAACAT GAACTAAATCTGGCAAGAAAAATGA GGGAATGGAAACTATGGGAGCCAT TAGTGGAAGAGCCTCCTGCCGATC AGTGGAAATGGCCAATATAATTATT AAGTGACTTTGGTGTGTTCATGGG AAACTGATGTAATTAAATATTCTGTT ATATT	Constitutive in Individual 3 and repressed in Individual 4	NM_005000

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
55	C + AP19	clone 717M23 on chromosome 20	363bp	CCAATTTTCTCAATTTTATTGAGTTA CCACCAAAAGTGTTAATTTAACAAT GTGATAGAGGTTGACAGTACGTTT CATTAGTACAAACATGCCTATGTTT TATATAAATTTATAGCTGATCAACTA GAGAATAAATATAATTATCCTTTTAT TTAGGTCTCTTTTACATTCACGTTT CTTTCACAGGTTGGAAAAACAAACC TTCACACAGGAATTGACAAATCAAA GGAATTGAGAAGCTCCTTACTGGC TGGTATCCATTTTTGGCACTTGTCA CAGAAGTAGGACAGCTGCTCCTCC ATCCAAGACACGTCTCCTTCATCAC TGTTGAGGGAATCCCCACTCTGGT CGAGCGATAAGCTT	Induced in Individual 3 and repressed in Individual 4	AL050321
56	C + AP20	FLJ22624 fis, clone HSI05951	85bp	AAGCTTGTTGTGCACAGGATCAAC ATGATGGTGACTGGGAAAAAATTA CTTCAAGTAACATGCTTAGCTTTCC CTCCTTAATGTG	Repressed in Individual 4, but not in Individual 3	AK026277
57	C + AP24	No significant match to anything	106bp	AGGGGTTTTACCAAGAGCATTTATT TACTCTTTAAGATACTAATGAAGAC CCTTAATATTGGTAAAATCATAACA TATAGGTTACATTATACAGCTAGNG AAGCTT	Induced in Individual 4, but not in Individual 3	
58	C + AP19	clone RP11-468G5	72bp	AAGCTTATCGCTCAATAGTGCATTC AACAGAAACTCTACAGGATTATACA CACAGTAGTAACACTGGCCTTG	Induced in Individual 4, but not in Individual 3	AC009238
59						
60	G + AP2	histone macroH2A1.2 (H2AF12M)	31bp	AAGCTTGCACCATGTACCCCACCT TCAGTTT	Increased in Individual 3, not in Individual 4	NM_004893

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
61	G + AP3	KIAA0949 protein	104bp	AAGCTTAACGAGGAATGAGAACAC AAGGAATGATTCAAGATCCACCTTG AGAGGAATGAACTTTGTTGTTGAAC AATTAGTGAAATAAAGCAATGATCT AAACT	Repressed in Individual 3, but induced in Individual 4	AB023166
62						
63						
64						
65						
66						
67	G + AP5	BCL2-related protein A1 (BCL2A1)	122bp	CGAGAAAAATACATACAATTTATTC ATTACATGGGGACAAAATTTCCATA ACTCTGGAAGGTCAAGTTACATCAT CAAAGTTGTTTATTTAAAAGTAGAA GTATGTGTTGGCAATCAAGCTT	Increased in Individual 4, but not in Individual 3	NM_004049
68		Insert is too small				
69	A + AP4	No significant match to anything	139bp	CACAATTTATTTATTGAGCTTTGCT GTATGTAGCTTTTTGAAGTAGATTT TATAGTTTAGAATTGTTGCTTTCCT TTTTTAATCTTTCACTCTTCATTTTT ATTGAGCTATGAATTAACTATTGTA GCGGTAAAAGCTT	Induced in Individual 4, but not in Individual 3	
70	A + AP4	chromosome 21, clone:KB51A8	123bp	AAGCTTTTACCGCTACTGAGTCTGT GTAGTAAATTTTTGACTAGGNACAT GGTGAGATTTACATTATTAAGTGTG AAGTTTTGTTAAGGTTCCTTTAAAG AATATTACGCTTTCTGGCCGGCG	Slightly induced in Individual 3, but not in Individual 4	AP001628
71	A + AP3	No significant match to anything	110bp	AAGCTTAACGAGGGATGGCAGCTG ATGGGTACCAGGTTCCTTTATGGG ATGATGAAAAAGCTCTGAAATTAGC TGTGGTGATGATAATTGCACAACTC CGTGGATATACG	Induced in Individual 4, but not in Individual 3	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
72	A + AP3	RECQL4 helicase	50bp	CCAAAGTGAGCATTTTTTATTCTGC ATTTTGGAGCCTCCTCGTTAAGCTT	Induced in Individual 3, but not in Individual 4	AB026546
73	G + AP4	Insert is too small			Induced in Individual 4, but not in Individual 3	
74						
75						
76	A + AP23	cyclin D2	179bp	AGGTCAAGGGGAGTTTATTGTCCA AATAGCATAACCTAATTGCATTCAA AACCATTTTCAAATCCATCTTTAAA CTAGTCANAAAACAGGTTATTATTT TTTTAAATCACTTANCACTGAACAG ATAAGACCTCTTAAAAGGCAGCTG ACTATATCATGTCACCATCATAGCC AAGCTT	Highly induced in Individual 4, but not in Individual 3 or Individual 5	NM_001759
77	A + AP23	clone RP1-63P18 on chromosome 1	124bp	AAGCTTGGCTATGGGTTGCCTAAA TTGATGTTTTGAGGAAGCATATTAA TGTTATAAACTTCGCTGACTTTGAA GGTNGTGTTGTAGCATGAGGANCA CAAATAAAACAATTCTAAATCAAAC T	Induced in Individual 4, but not in Individual 3 or Individual 5	AL356379
78	C + AP19	No significant match to anything	38bp	CTAGAAAGCAGACACTCCATAACC TGAGCGATAAGCTT	Induced in Individual 4 and Individual 5, but not in Individual 3	
79	C + AP19	No significant match to anything	36bp	AAGCTTATCGNTCAGGTTATGGAG TGTCTGCTTTCT	Induced in Individual 4 and Individual 5, but not in Individual 3	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
80	C + AP24	cytochrome c oxidase subunit VIIc (COX7C)	213bp	CACGTCATAAGTTTATTGACAAACA TATCTAGTATGGCATATGAGTTCTA GTTTGATCCACTTCCAGAGGCTGC ACCTCTTAAAATGCTCTTCATATCT GTTAAATGGAGGAACTGAAACATC CTTATGTTTTAAGCAGTTGGTGTCT TACTACAAGGAAGGGTGTAGCAAA TGCAGATCCAAAGTACAAACACATC TTAGCTAGTGAAGCTT	Induced in Individual 3, but not in Individual 4 or Individual 5	NM_001867
81						
82	C + AP24	clone RP11-358M9	146bp	CAGTTAAAAAGAAATGGTAGACAC CTATATTTACTTTGTTTAGACATACA AAGGCTAGCCTCTTTTGACTTGTAC AAAGTTTTTCAAACTTTCATATACAA ATATGCCCACTCATTTATTCATTCA TCGGACAGCTAGTGAAGCTT	Very high expression in Individual 4 and Individual 5, but not in Individual 3	AC020595
83	C + AP19	Human mitochondrial DNA	35bp	AAGCTTATCGCTCACACCTCATATC CTCCCTACTG	High expression in Individual 3, but not in Individual 4 or Individual 5	NC_001807
84	C + AP1	ribosomal protein L3	64bp	AAGCTTGATTGCCAGGAACAGATTT TGCAGTTGGTGGGGTCTCAATAAA AGTTATTTTCCACTG	Slightly induced in Individual 3, but not Individual 4 or Individual 5	M90054

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
85	C + AP8	No significant match to anything	239bp	AAGCTTTTACCGCTAAAATGATGAT ACANGTTGAAGACACATCACTCTG AAATTGGAAGACCTCACCACTTAAG GCTCCACAGTGGCTTACTCAGCTG AACTCTAGGTTACTACTCTTTACTT TGTNCACCCATNGGGGGGTGCAGT TTTTTTAAAATGTTGGGAGATGGCC ATTCTAACTACTGTTGAATGTCTCT GTTTTGGGAAGGTATAACAAGAAAT AAAAAAGANTATATATG	Induced in Individual 4, but not in Individual 3 or Individual 5	
86	C + AP19	GTP-binding protein G25K	266bp	AAGCTTATCGCTCCAAAGACTGCT GAAAAGCTGGCCCGTGACCTGAAG GCTGTCAAGTATGTGGAGTGTTCT GCACTTACACAGAGAGGTCTGAAG AATGTGTTTGATGAGGCTATCCTAG CTGCCCTCGAGCCTCCGGAAACTC AACCCAAAAGGAAGTGCTGTNTATT CTAAACTGTTTTCTCCTTCCCTTCT TTGCTGCTGCTTCCTGTCCCACTA CTGNAGAAAGATCGTTTAAAAACAA AGGAATAAAACCNTCCTGTTTG	Induced in Individual 3, but not in Individual 4 or Individual 5	M35543

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
87	C + AP36	ribosomal protein S4, X-linked (RPS4X)	332bp	AAGCTTCGACGCTGGTAACCTGTG TATGGTGACTGGAGGTGCTAACCT AGGAAGAATTGGTGTGATCACCAA CAGAGNGAGGCACCCTGGATCTTT TGACGTGGTTCACGTGAAAGATGC CAATGGCAACAGCTTTGCCACTCG ACTTTCCAACATTTTTGTTATTGGC AAGGGCAACAAACCATGGATTTCT CTTCCCCGAGGAAAGGGTATCCGC CTCACCATTGCTGAAGAGAGAGAC AAAAGACTGGCGGCCAAACAAAGC AGTGGGTGAAATGGGTCCCTGGGT GACATGTCAAATCTTTGTACGTAAT TAAAAATATTGTGGCAGG	Slightly induced in Individual 3, but not Individual 4 or Individual 5	NM_001007
88	G + AP1	clone RP1-68D18 on chromosome 11p12-13	231bp	GGTCAAGACACCTTCAGAAAAATG TTAGAATCTACACCTACGTCAGTCA TTTCATTGTGTTCACAATTTGCTAA TTCATTTTTTATCTTCTCTAGTATCA GATCAGATCAGTGTACCTCCAAAC AGAGATGGAAGCTACACTGCAGTT CCCAATACTACTTCAGCATAGAGCA AAAATGTGAAGCCAATTAACAGAGA AATCATTTTTGGCATTATTAGGCAA TCAAGCTT	Very high expression in Individual 4 and Individual 5, but not in Individual 3	AL133330
89	G + AP36	Human mitochondrial DNA	28bp	GCGGGCGGGGGATATAGCGTCGA AGCTT	Induced in Individual 5, but not in Individual 3 or Individual 4	NC_001807
90						

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
91	C + AP39	ecto-ATP diphosphohydrolase	113bp	AAGCTTTCCCAGCTACACCTTTCTC CTTTGTACTTTGTGCTTGTATAGGT TTTAAAGACCTGACACCTTTCATAA TCTTTGCTTTATAAAAGAACAATATT GACTTTGTCTAG	Induced in Individual 3, but not in Individual 4 or Individual 5	AJ133133
92	C +AP3	ATPase, H+ transporting, lysosomal (vacuolar proton pump) 9kD (ATP6H)	131bp	AAGCTTTGGTCAGTAGGAGAGCAC GTTCAGAGGGAAGAGCCATCTCAA CAGAATCGCACCAAACTATACTTTC AGGATGAATTTCTTCTTTCTGCCAT CTTTTGGAATAAATATTTTCCTCCTT TCTATGG	Induced in Individual 3, but not in Individual 4 or Individual 5	NM_003945
93	A + AP3	cyclin D2	265bp	AGGTCAAGGTGAGTTTATTGTCCAA ATAGCATAACCTAATTGCATTCAAA ACCATTTTCAAATCCATCTTTAAAC TAGTCAGAAAACAGGTTATTATTTT TTTAAATCACTTAACACTGAACAGA TAAGACCTCTTAAAAGGCAGCTGA CTATATCATGTCACCATCATAGCCA ATACAACATTTTTGCCATACTTCCT AAAAACCTTTTCGCATACACTGATC ATGCTACTTATCAGCACTTTCTAAC ATCCTGACCAAAGCTT	Induced in Individual 4, but not Individual 3 or Individual 5	NM_001759
94		no insert - just vector				
95		No significant match to anything	122bp	AAGCTTCGACGCTGGGACACACAA GAACAGAGAAGTAAAAGATTTAAGT GTTCTGACATTTCCTACAATGATCT TGGCTTCTGTACCAATTGTTTTTCT CCTACCAAGTACTTGCTCAGTGT	Induced in Individual 1, but not in Individual 6 or Individual 7	
96						

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
97	A + AP8	inhibitor of apoptosis protein-1 (MIHC)	310bp	ACCAGNGGTTTGCAAGCATGGTTT CCTGGATCTNACAGACGTTGGGCT TTTCAACTGCAGACTTCAATGCTTT GTTTCCAGGTGGCAGGANAAACAT CACATCTCATTACCAACAATTATTT CCCTCAAAGGTAAGTAATTGTATCT AACTTCAATATTCCTTTCCCTGGCC AAAAATTTTACACATTGTTATAAAAA TCTTCTTTGTATTCATCACTGTCTTT ATATTAATTTTCTAAGTTTCTTCATT TCCTAAAATGTAACCTTCAACCACC ATTCTCATCCTCATCATTCTCAGCG GTAAAAGCTT	Induced in Individual 7, but not Individual 6	AF070674
98	A + AP8	ribosomal protein L35a (RPL35A)	441bp	ACAAGAGCACAAATCCACATTTATT TATTGATTTTTCGTTAGTTTAAATCC TTGAGGGGTACAGCATCACTCGGA TTCTGTGTCCAATGGCCTTAGCAG GAAGATTGCTTCGGAATTTGGCAC GAACCATGCCACTGTTTCCATGGG CCCGAGTTACTTTTCCCCAGATGA CTCTGGTTTTGTTTGGTTTGCCGCC AGGAGTGACTGTGTTGTTCTTTGCT TTATATACATAAGCGCATCTCTTGC CCAAATAGAATTCTGTTTCATCTCG GGCGTAAACACCTTCAATTTTAAGA AGAGCTGTGTGCTCCCTTTGGTTC CGGAGACCCCGCTTATAGCCAGCA AAAATGGCCTTGGACCACAGCCTT CCAGACATAGTTCCTTTTAGAAAGT CCCGTTCCCAGCAGGCCTCCACAG GAGCCAAGATGGCGGTAAAAGCTT	Induced in Individual 7 and not in Individual 6	NM_000996

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
99	G + AP10	ribosomal protein S12	443bp	AAGCTTCCACGTAACCCACCGCCA TGGCCGAGGAAGGCATTGCTGCTG GAGGTGTAATGGACGTTAATACTG CTTTACAGGAGGTTCTGAAGACTG CCCTCATCCACGATGGCCTAGCAC GTGGAATTCGCGAAGCTGCCAAAG CCTTAGACAAGCGCCAAGCCCATC TTTGTGTGCTTGCATCCAACTGTGA TGAGCCTATGTATGTCAAGTTGGT GGAGGCCCTTTGTGCTGAACACCA AATCAACCTAATTAAGGTTGATGAC AACAAGAAACTAGGAGAATGGGTA GGCCTTTGTAAAATTGACAGAGAG GGGAAACCCCGTAAAGTGGTTGGT TGCAGTTGTGTAGTAATTAAGGACT ATGGCAAGGAGTCTCACGCCAAGG ATGTCATTGAAGAGTATTTCAAATG CAAGAAATGAAGAAATAAATCTTTG GCTCAC	Very high expression in Individual 1 and Individual 7, but not Individual 6	NM_001016
100	G + AP10	Thymidylate synthetase	196bp	GCAGAACACTTCTTTATTATAGCAA CATATAAAACAACTATAAAGTTCAT AACCACACTCTACATCATGATCGAT GGTGTTACTCAGCTCCCTCAGATT NGAGGGAATAGCTNGTGAAATTCT TAAAATATTCTAAAAATATTCCAAAA ATAGCTNGTGAAATNCACCAACCTT CTTTATAAGTACGTGGAAGCTT	High expression in Individual 6, but not in Individual 1 and Individual 7	NM_001071

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
101	G + AP12	chromosome 17, clone hCIT.211_P_7	314bp	GGAAGTAGTATATTTCATTTTATTAT AAAGCAGTGCTCCCAAACTTTTCAC AGCGTACACCTNGAGGGTGGAGAA CATCCAACCNCACCTGGATGGTGG ATGGGACCCACTTCTGGGTAACCT GATGAGGAAGCTCTAGTGTANAAA TTCAGGACGCGGTCTTCAAAGCAG AGGGCTTGGTTCAAGTCCCTGTTC TGCCACTTACTAACTGCATGACCTT GAGCAAGCCACTTAATTTCTCTGCT CCTTCTCTGTGAAATGGGTACAATG TGGTCAGCAGTAAAGGAACTAATA CACGTACAGCACTCAAGCTT	Higher expression in Individual 1 and Individual 7 than in Individual 6	AC003665
102	G + AP19	just vector				
103	G + AP19	just vector				
104	A + AP3	cyclin D2	265bp	AAGCTTTGGTCAGGATGTTAAAAAG TGCTGATAAGTAGCATGATCAGTGT ATGCGAAAAGGTTTTTAGGAAGTAT GGCAAAAATGTTGTATTGGCTATGA TGGTGACATGATATAGTCAGCTGC CTTTTAAGAGGTCTTATCTGTTCAG TGTTAAGTGATTTAAAAAAATAATAA CCTGTTTTCTGACTAGTTTAAAGAT GGATTTGAAAATGGTTTTGAATGCA ATTAGGTTATGCTATTTGGACAATA AACTCACCTTGACCT	Induced in Individual 1, but not in Individual 6	NM_001759
105						
106		just vector				
107		just vector				

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
108	C + AP16	clone DKFZp564O092	355bp	AAGCTTTAGAGCGTCAGTAAGCGA GAGAAAGGACGGCGAAAACGAGCA AATGTCATGAGCTCACAACTTCATT CCCTTACACACTTCAGTGACATCAG TGCTTTGACAGGGGGAACTGTTCA TCTTGATGAGGATCAGAATCCTATT AAGAAGCGGAAGAAGATACCTCAG AAAGGTCGGAAGAAAAAAGGTTTT CGGAGGCGGCGGTGATTATGGGT GTACATATTTGTATATTTTTTGTCAT CCTGAGATACTTCTAATTTCATTGT ATATAGGTGGTTTTCCCTGGAATTC ATTAATTGTTTGCTTTGGACATGTG GAAAGAGCCTTACTAATAAAATTGA TTTTACTTATG	Strongly induced in Individual 1 and not in Individual 6	AL050003
109	C + AP9	cDNA FLJ11508 fis, clone HEMBA1002162	150bp	CCATCAAATGTAATTTATTTAAATAA CAATTCAATTGCATGTTAAGTAAAC CAGTTGTAGCAATATAAAAATACAG AATTTTGAGAAAATCTGGCAAATTA AACCTGTATCTAAATGCAGCATATT CTGTGATACTACGGAATGAAGCTT	Slightly induced in Individual 1 and not Individual 6	AK021570
110	A + AP22	mitochondrial genomic DNA	64bp	AAGCTTTTGATCCAAGCCTACGTTT TCACACTTCTAGTAAGCCTCTACCT GCACGACAACACAT	High expression in Individual 6, but not in Individual 1 or Individual 7	NC_001807
111	A + AP20	NADH dehydrogenase-ubiquinone Fe-S protein 2 precursor	69bp	AAGCTTGTTGTGCATGTACTAAAAA AGGAGAAATTATAATAAATTAGCCG TCTTGCGGCCCCTAGGCCT	High expression in Individual 6, but not in Individual 1 or Individual 7	AF013160

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
112	C + AP9	No significant match to anything	165bp	AAGCTTCATTCCGAGGGTACCGTT TTAGGCTCTTATTGACCATTCTGAA CAGCGTGCTGTCTTTTCTTTGGTTC AAGTTTCTCTGGCTAACCTCTTTAA TGTTTCCTTTTAAATGAAAACTTGG CTTTCGTTTCCAACTTTCCCTCTGG GCATGGCTCTTTTTGG	Induced in Individual 1, but not in Individual 6 or Individual 7	
113	C + AP9	cytochrome b gene	233bp	AAGCTTCATTCCGATAAAATCACCT TCCACCCTTACTACACAATCAAAGA CGCCCTCGGCTTACTTCTCTTCCTT CTCTCCTTAATGACATTAACACTAT TCTCACCAGACCTCCTAGGCGACC CAGACAATTATACCCTAGCCAACC CCTTAAACACCCCTCCCCACATCA AGCCCGAATGATATTTCCTATTCGC CTACACAATTCTCCGATCCGTCCCT AACAAACTAGG	Induced in Individual 1, but not in Individual 6	AF254896
114	C + AP11	just vector				
115	C + AP10	lysosomal pepstatin insensitive protease (CLN2)	157bp	AAGCTTCCACGTAGTATTGAAATGG CCAGTTTACTTGTCTGCCTTCCTTT CCAAGACCGTTGGTGCCTAGAGGA CTAGAATCGTGTCCTATTTAACTTT GTGTTCCCAGGTCCTAGCTCAGGA GTTGGCAAATAAGAATTAAATGTCT GCTACACCG	Expression in Individual 6, but not in Individual 1 or Individual 7	AF039704
116	C + AP19	clone 108K11 on chromosome 6p21	52bp	CAAACAAAATTCTTTACCTCATTTC TCGTTCTTCATGTTGAGCGATAAGC TT	Induced in Individual 7, but not in Individual 6	Z85986
117	C + AP19	No significant match to anything	117bp	AAGCTTATCGCTCACACTGTCATGT TGAATTTAACCTGTGGCTTTGTAAA ACAAGAAGGAATGAAATTATACTGA AGTAAAGTCTGCAGTTATGCCCCA AATGAGATGACCCCTTGG	Induced in Individual 1, but not in Individual 6	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
118	C + AP19	No significant match to anything	213bp	CTAGGTCTCTTTCCAGTCTTTCTAC CTCTTCTAAACTGAATCTCTCAAAA GCCTCCTGTCTATCCTGAGTGGTT ACTGGCTGACCTTCCAAACTTTCCA ATGAACGGAGGTGGAAAATGGTAA ACTGGAGGTAATGAGGAAGGGTCA CAACTGGATTTTCAACTAGGATCAG AGAAATCAAATCTTGAAGCAGTTTC AACTTGCTTATATCTT	Repressed in Individual 6, but induced in Suzannna	
119	C + AP23	No significant match to anything	66bp	AAGCTTGGCTATGGAAGAATGAAT AGCAAAAAAAGGAGAATTTTTTTAA AAAGATCTCTCACTGGG	High expression in Individual 6, but not in Individual 1 or Individual 7	
120	C + AP22	mitochondrial ATPase coupling factor 6 subunit (ATP5A)	366bp	CAACATCACCAAATAATTTATTTGG ACTCAGAATTAAAAGAACATTTGAC AGTTATGAAATGCATGTTTATTCTG AAACTTCTAACTAGTTGTACAACTA ATCCGTGACAAATTACCAGATTAAT TTTACTTTATTTCTTCAGGCCTGGG GTTTTTCGATGACTTCAAATTTGGG ATCTTCAAATTTGAAGGTGGGAAAT GTATTCATGTCTGCATTACCAAACA TTTGCTTGAGCTTAAAAAGCTCCCT CTCCAGCTCTTGCTGATACTCTGAA CTAGCATCAACAGGTCCTCCAGAT GTCTGTCGCTTAGATTTGTATTCTC TAATCTTGTCCACAAAGAGTTTCTG TATAGGATCAAAAGCTT	Induced in Individual 7, but not Individual 6	M37104

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
121	C + AP19	clone 717M23 on chromosome 20	363bp	CCAATTTTCTCAATTTTATTGAGTTA CCACCAAAAGTGTTAATTTAACAAT GTGATAGAGGTTGACAGTACGTTT CATTAGTACAAACATGCCTATGTTT TATATAAATTTATAGCTGATCAACTA GAGAATAAATATAATTATCCTTTTAT TTAGGTCTCTTTTACATTCACGTTT CTTTCACAGGTTGGAAAAACAAACC TTCACACAGGAATTGACAAATCAAA GGAATTGAGAAGCTCCTTACTGGC TGGTATCCATTTTTGGCACTTGTCA CAGAAGTAGGACAGCTGCTCCTCC ATCCAAGACACGTCTCCTTCATCAC TGTTGAGGGAATCCCCACTCTGGT CGAGCGATAAGCTT	Gene expressed at same level in everyone	AL050321
122	C + AP19	clone RG013F03	126bp	CGAAAATAGAAAAATGTATTTACAA GTAGTACGTTACTATGATGAGAAAG CACAAAGACACCTGCTGCTATAATA CATGCATTGGCTCGAGAAGAAACT ACGGAACACCCTGCGAGCGATAAG CTT	Repressed in Individual 1, but not in Individual 6	AC005046
123		just vector				
124	G + AP10	thymidylate synthetase	196bp	GCAGAACACTTCTTTATTATAGCAA CATATAAAACAACTATAAAGTTCAT AACCACACTCTACATCATGATCGAT GGTGTTACTCAGCTCCCTCAGATTT GAGGGAATAGCTTGTGAAATTCTTA AAATATTCTAAAAATATTCCAAAAAT AGCTTGTGAAATTCACCAACCTTCT TTATAAGTACGTGGAAGCTT	High expression in Individual 8 and Individual 4, but not Individual 9	NM_001071

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
125	G + AP19	clone RG013F03	126bp	AAGCTTATCGCTCGCAGGGTGTTC CGTAGTTTCTTCTCGAGCCAATGCA TGTATTATAGCAGCAGGTGTCTTTG TGCTTTCTCATCATAGTAACGTACT ACTTGTAAATACATTTTTCTATTTTC C	High induction in Individual 4, but not in Individual 9	AC005046
126	G + AP19	clone RG013F03	126bp	GGAAAATAGAAAAATGTATTTACAA GTAGTACGTTACTATGATGAGAAAG CACAAAGACACCTGCTGCTATAATA CATGCATTGGCTCGAGAAGAGACT ACGGAACACCCTGCGAGCGATAAG CTT	High induction in Individual 4, but not in Individual 9	AC005046
127		just vector				
128	C + AP9	clone YB26B05	276bp	CAGGAGGCATGCAGGGAGGACAC TGCAGATGATCAAAGTTTTATTTAA CATTATAGAACACTTAAAAATAAAC AATATGATTGCATTTCTGTTGTGTA ACTTTGAAAAATGTTTTCAAGCACA CATAAAAGCAACAAGTTTTACCCCC AACAAGTATGCTATGCAGCCAGTC CCTAACTGCTGGGGTCAGCACTGC TCTCAACAGACAGCTGGGGTAGGA GGTAGGCTGCTGGTGTCCCTGCAG GCAGGTGGCTTTTCCTACTCGGAA TGAAGCTT	Repressed in Individual 8 and Individual 4, but not Individual 9	AF147336

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
128	C + AP9	uncoupling protein-2 (UCP2)	267bp	AAGCTTCATTCCGCTCCTTTACCTA CCACCTTCCCTCTTTCCCCACCTCT TCCTTCCGCTCCTTTACCTACCACC TTCCCTCTTTCTACATTCTCATCTA CTCATTGTCTCAGTGCTGGTGGAG TTGACATTTGACAGTGTGGGAGGC CTCGTACCAGCCAGGATCCCAAGC GTCCCGTCCCTTGGAAAGTTCAGC CAGAATCTTCGTCCTGCCCCCGAC AGCCCAGCCTAGCCCACTTGTCAT CCATAAAGCAAGCTCAACCTTGG	Repressed in Individual 8 and Individual 4, but not Individual 9	AF096289
129	C + AP16	Cide-b (CIIndividual 3)	385bp	AAGCTTTAGAGCGGAGGGTTAGCA GTCACCTGAGTAAGTCACTGGGGT TCAGAGCTGAGAGGTACTCCATGG TGGACCGGAGAGTTCCTTCCCTGG AACTTCTGGGCTGGGTGGTTCTCT CCTGTGCTGGGGCTTTAGTGGTGT TTTCTGTTACAAACCTGGGATCTCA GCCCAGGACAAGGTGGGAATGAGT CAAGCCTGGACTCTGGCCCCCCTG CCTGGCCAGTAAGAAGGGCAAAGT CCAAGGGGAGGGATGAGGGAGGG GCCAGATGGGGTCCTGGAGGAAG AATTGCCTGGCAAAAGCCATTGGA GCTTGTATGTGTGTCTTTGGTGATG ACATGTGTTGTGAGGGTAGATGGG AACCATGTAAAAGGATGAAATGTG	Induced in Individual 9, but no in Individual 8 or Individual 4	AF218586

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
129	C + AP16	No significant match to anything	385bp	CTGGGAATAAAACTACATCTCAAAA CCTCTTTGCAACCGAGTGTGAGTA TGTTTTCACCAATAAGACATAAGCA GATAGAGGATGCAAGTCACTTCCT AACAAACATCTTTATAAGCCAGCTG ACACATACCCATTCACCATATCATA GGTTATGTAGTCTCAGGTTGCTGC AGAGTAATGTCCTCTTTTAGACATG GTAGAGAATTTATGTACAGTGGAG CAGAGACTGCAGCAACTACAGACT TCAGCCACATAACTTACTTCTCTAT TATAAGACAGCAAGAAAACAGACTT ATCTTCCAGAGTCACACAGCTGGT ATCCAGAGAAGGAGAAAGGGATAC AAGTATGTGCACTCTGAATATGAAC ACTACGCTCTAAAGCTT	Induced in Individual 9, but not in Individual 8 or Individual 4	
130	G + AP43	mitochondrial DNA for D-loop	230bp	GGGTATGCACGCGATAGCATTGCG AGACGCTGGAGCCGGAGCACCCT ATGTCGCAGTATCTGTCTTTGATTC CTGCCTCATCCTATTATTTATCGCA CCTACGTTCAATATTACAGGCGAAC ATACTTACTAAAGTGTGTTAATTAAT TAATGCTTGTAGGACATAATAATAA CAATTGAATGTCTGCACAGCCGCT TTCCACACAGACATCATAACAAAAA ATTTCCAC	Induced in Individual 1, but not in Individual 6	AJ230610

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
130	G + AP43	no significant match to anything	260bp	GAAATTTTGATTAAATTCTTTATATC ATTCTTGCAACTTTTCCATGCTTTT GAAATTATTTAAGAAAGTTAACAAA TAAGCAAACAAACTACAAATCAGGA ATGGAAAGTGAATTTTACAAAATGC CATTTTCAGATTCACAAGACGCTTG CATTCACCTTCCTTCGACGATTACT ACTTCTTACACGCTATGATTTCTCT CCTTTTTCCACCACACCGGCTCCA GGTCTACTTGTCTTTACTCTCCCCG CTTCAAGCTT	Induced in Individual 1, but not in Individual 6	
131						
132						
133		No insert - just vector				
134	G + AP47	chromosome 10 clone RP11-70E21	257bp	GAGATAGAGTCTTGCTGTATCACCT AGGCTGGAGTGCAGTGGCATGATC ATAGCTCACTGCAGGCTCAAACTC TTAGGTTCAGATGATTCTCCCACCT CAGCCTTCTGAGTAGCTGAGATTA CAGGTGTGTATGACTATGCCTGGC TAATTTTTTTTTTGTAAAGATGGGG TCTTGCTATGTTGCCCAGGCTGGT CTCAACCTCCTGGCCTCAAGTAAT CCTCCTGCCTCGGCTTCTGGAATT ACGGGCATAAGCTT	Induced in Individual 1, but not in Individual 6	AC016399
135	A + AP41	matches a bunch of sequences that make no sense	158bp	ATATACTTTAAGTTCTAGGGTACAT GTGCACAACGTGCAGGTTTGTTAC ATATATATATACATGTGCCATGTTG GTGTGCTGCACCCATTAACTCGTC ATTTACTTTAGGTATATCTCCGAAT GCTATCCCTCCCCTCTCCCCCCAC CCCGTAAGCTT	Induced in Individual 1, but not in Individual 6	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
136		jun B proto-oncogene (JUNB)	112bp	ACTTAAATAGATTCAATAAAAAGAA CAAACACACACAAACACAAACACG TCTTAAAATAAACTCTTTANAGACT AAGTGCGTGTTTCTTTTCCACAGTA CGGTGCAAAGCTT	Induced in Individual 1, but not in Individual 6 or Individual 7	NM_002229
137		Chromosome X clone bWXD501	63bp	AATAAGAACCAAAAGAAATTTATTT ACAAATTTCTTACTTGACCAGTAGT GTCAGCCAAGCTT	Induced in Individual 1, but not in Individual 6	AC004677
138		No insert - just vector				
139	A + AP47	Chromosome 16 BAC clone CIT987SK-A-67A1	106bp	AGATATAGAAGTCCCACTAGGTTG CCCTGGCTGGTCTTAAACTCTTGG TTTCAAGTGATCCTCCTGCCTTGG CCTTCCAAAATGCTGGTATTACGG GCATAAGCTT	Induced in Individual 7, but not in Individual 6	AC004531
140	A + AP47	Chromosome 16 BAC clone CIT987SK-A-67A1	106bp	AAGCTTATGCCCGTAATACCAGCAT TTTGGAAGGCCAAGGCAGGAGGAT CACTTGAAACCAAGAGTTTAAGACC AGCCAGGGCAACCTAGTGGGACTT CTATATCT	Induced in Individual 7, but not in Individual 6	AC004531

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
141	C + AP41	No significant match to anything	464bp	CAGGGGAGGGGGACACCACTCAA ACTATTAAAACCTTTACAGCAACGT GACCGTGGATAAATTATACAATTAT AAGTGCTGAGGATTCTTAATAAAAA ATATAAGGATTTGAAATTAAATATTT CAAACATCAAGAAAATAGACACATA TGTAACAATAATCCAGACTAAAGAA ATGTCAGCATTTGTCATATTTATTA CATTTTTTAACTTTTTAAAAAAATGA GATATTATAGATAAAACAACTCTCC ATCTCATTCTCCTCCTACCTTCTCC AAATATAGTCACTATTCCTGAAGTA TGTTTGCATTCATAGCAATTTTATTA TTTTAGTATTGCATACTTGATGATG CAAGTAAAACTAACTTATTTTAACT GCTACATCAGAGACTTTAGAATTGA AATTGACTTCTCTGAGCCTCAGAAT CTTTCATAATATTCTTGCCCTAGCC ACCCCGTAAGCTT	Induced in Individual 1 and Individual 7, but not in Individual 6	
142	C + AP41	Fc-gamma-receptorIIA (FCGR2A)	240bp	AAGCTTACGGGGTCATACTACATA CAAGCATAAGCAAGACTTAACTTG GATCATTTCTGGTAAATGCTTATGT TAGAAATAAGACAACCCCAGCCAA TCACAAGCAGCCTACTAACATATAA TTAGGTGACTAGGGACTTTCTAAGA AGATACCTACCCCCAAAAAACAATT ATGTAATTGAAAACCAACCGATTGC CTTTATTTTGCTTCCACATTTTCCC AATAAATACTTGCCTGTG	Induced by penicillin in Individual 1, but not in Individual 6	M90727
143	C + AP45	Chromosome X clone bWXD501	67bp	CGATAATAAGAACCAAAAGAAATTT ATTTACAAATTTCTTACTTGACCAG TAGTGTCAGCCAAGCTT	Induced in Individual 7, but not in Individual 6	AC004677

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
143	C + AP45	clone CTA-407F11 on chromosome 22q12	64bp	AAGCTTGGCTGACAATCTCAGAGG TCACCTTTATAGAAAAATATAGTCT AGGCTTAGGTTCCTG	Induced in Individual 7, but not in Individual 6	AL022329
144	C + AP48	3' end of PAC 92E23 containing the X inactivation transcipt (XIST) gene	104bp	CAAAACAGTATATTTTATTTTACAAT AGCAACCAACTCCCCAGTTTGTTTC AATTGTGACATCTAGATGGCTTAAG ATTACTTTCTGGTGGTCACCGCAA GCTT	Repressed in Individual 1, but not in Individual 6	U80460
145	G + AP53	SH3-containing protein EEN (EEN)	253bp	GTAGAAAGAGACATTTAATACTTCT GTTTACAAAATTCAGGCGTACATTT CAGTTTGCCCTGNACCGTGCCCAA AGCTGTGTGCTCATCTCTGCGCCC CTCATGTACTTCTGACGAGGGGGG TGCAGGGCAGGGCAGAGCAGAGC CTGGGGTCCGGAGGCTTCACTGGA CCACAGAGGGAGGGGAATGTGAAT GTGGCCTGCCCAGAGAACTCCCCA TTTCATCGATTTTGCATTGGGCGAT AGAGGAAGCTT	Induced in Individual 6, but not in Individual 1 or Individual 7	AF190465
146	G + AP53	SH3-containing protein EEN (EEN)	255bp	GTAGAAAGAGACATTTAATACTTCT GTTTACAAAATTCAGGCCGTACATT TCAGTTTGCCCTGGACCGTGCCCA AAGCTGTGTGCTCATCTCTGCGCC CCTCATGTACTTCTGACGAGGGGG GTGCAGGGCAGGGCAGAGCAGAG CCTGGGGTCCGGAGGCTTCACTG GACCACAGAGGGAGGGGAATGTG AATGTGGCCTGGCCCAGAGAACTC CCCATTTCATCGATTTTGCATTGGG CGATAGAGGAAGCTT	Induced in Individual 6, but not in Individual 1 or Individual 7	AF190465
147						
148						

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
149	G + AP55	No significant match to anything	287bp	GTAAATTTGTTTGAGTTCATTGTAG ATTCTGGATATTAGCCCTTTGTCAG ATGAGTAGGTTGTGAAAATTTTCTC CCATTTTGTAGGTTGCCTGTTCACT CTGATGGTAGTTTCTTTTGCTGTGC AGAAGATCTTTAGTTTAATTAGATC TCATTTGTCAATTTTGTCTTTTATTG CCGTTGCCTTTGGTGTTTTGGACAT GAAGTCCTTGCCCATGCCTATGGC CTGAATGGTAATGCTTAGGTTTTCT TCTAGGGTTTTTATGGTTTTATGTC TAACGTAAGCTT	Induced in Individual 1, but not in Individual 6	
150	G + AP54	ribosomal DNA complete repeating unit	215bp	AAGCTTTTGAGGTCAGGAGTTCGA GACCAGCGTGGCCAACGTGGAGA AACCCCGTCTCTACTGAAAATAGG AATATGAGCCGGCCGTCATGGTGT GCGCCTGTAATCCCAGCTACCGAA GAAGAATCACTGGAACCCGGGAAG CAGAGGTTTCAGTGAGCCGAGAGA GCGCCACCGCACCGCAGCCTGGG TGACAGAGCGAGAGAGACTCAGTC C	Induced in Individual 1, but not in Individual 6	U13369
151	A + AP52	heat shock 60kD protein 1 (chaperonin) (HSPD1)	241bp	AAGCTTGACCTTTATTAATGAACTG TGACAGGAAGCCCAAGGCAGTGTT CCTCACCAATAACTTCAGAGAAGTC AGTTGGAGAAAATGAAGAAAAAGG CTGGCTGAAAATCACTATAACCATC AGTTACTGGTTTCAGTTGACAAAAT ATATAATGGTTTACTGCTGTCATTG TCCATGCCTACAGATAATTTATTTT GTGTTTTTGAATAAAAAACATTTGT ACATTCCTGATACTGGGT	Induced in Individual 6, but not in Individual 1 or Individual 7	NM_002156

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
152	A + AP52	cDNA FLJ20436 fis, clone KAT03972	204bp	AGGGGAGTGTGCTAGTACAGAGGA ATCCTTTTATGATCAACTAGAGTCT GGGTCCTTCACTTTCAGTCGAGTG CTGTCTCCATTAGACCACAGTAAAG AGTTAACAGGTCACCAATGGCAGT CGCATCTTTCTGGGTAAGGCAGTG TCTGGTCATTGGAAGAGACTGATT GGAACAACGAACATCATCCACAAA GGTCAAGCTT	Induced in Individual 6, but not in Individual 1 or Individual 7	AK000443
153	C + AP55	No significant match to anything	407bp	AAGCTTACGTTAGAACCTGCAATAT TCTCGTGTTGTGTTGTCTTCTCTTT CCTGTCACAGTGTGTTATGCTTCC CAGCAGCAGACACGTATGTGTGTA CACACATACTCATTCATTTATTCATT TTAGGAGAAGGCATTCACACATGA AAAACAATTGAGATTAATGGTAGTG TTTTAGGACCAGTGGCTTGGGGGC TTTTTATGGGTATTCCTAGCACCCA TAAAGCAATTTAGGAATTAACCATT CCCTTTCCTAATAGGATACCAGCCT TGAGAGAAGTGTTGGTCATATTTCT ACAGCCTCATAATAGTAAAGTCAGT GTCTTCATCACATGGACTCAGCATA CTCTTAGAACTCCTCTTTAGTGGGT CAAATCTAGATNTACTTGCCCCCAT TTTATTATTG	Repressed in Individual 1 and Individual 7, but not in Individual 6	
154	A + AP47	Sid3177 mRNA (this is a mouse gene)	95bp	AAGCTTATGCCCGGGCTGAAAACC TCAATTTATGTTCATGACAGTGGGG ATTTTTTTAAATGTCTACATTCTTTC TAATAAACTGTTGGAAGACT	Induced in Individual 8 and Individual 10, but not in Individual 9	AB024935

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
155	A + AP47	Chromosome 16 BAC clone CIT987SK-A-67A1	106bp	AAGCTTATGCCCGTAATACCAGCAT TTTGGAAGGCCAAGGCAGGAGGAT CACTTGAAACCAAGAGTTTAAGACC AGCCAGGGCAACCTAGTGGGACTT CTATATCT	Induced in Individual 10, but not in Individual 9	AC004531
156						
157	G + AP41	ribosomal protein L8	218bp	GGCATAAACACAAACTTTATTGAGG CCCTCAGCACTAGTTCTCTTTCTCC TGTACAGTCTTGGTTCCCCGGAGA CGTCCAGTCCGGCGGGCAGCAAT GAGACCCACTTTGCGGCCAGCAGG GGCATCTCTGCGGATGGTGGAGG GCTTGCCGATGTGCTGGTGGTTGC CACCTCCAAAAGGATGCTCCACAG GATTCATGGCCACACCCCGTAAGC TT	Induced in Individual 10, but not in Individual 9	NM_000973
158	G + AP41	tapasin (NGS-17)	58bp	GGAAGCACTGGAATACAGCTTTATT CCTACACGATTAGACCCGTTACCC CGTAAGCTT	Induced in Individual 10, but not in Individual 9	AF029750
159	G + AP47	CoREST protein (COREST) (KIAA0071 protein)	149bp	AAGCTTATGCCCGGAAACAAAAAC GGGAGCGGGAGGAGAGCGAGGAT GAACTGGAAGAGGCAAATGGAAAC AATCCCATTGACATTGAGGTTGATC AAAACAAGGAAAGCAAAAAGGAGG TTCCCCCTACTGAGACAGTTCCTC AGGTC	Induced in Individual 10, but not in Individual 9	AF155595
160	G + AP43	mitochondrial DNA hypervariable II region	104bp	GTAGGACATAATAATAACAATTGAA TGTCTGCACAGCCGCTTTCCACAC AGACATCATAACAAAAAATTTCCAC CAAACCCCCCCCTCCCCCCGCTTC AAGCTT	Induced in Individual 10, but not in Individual 9	AF278478

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
161	G + AP52	clone RP11-517O1 on chromosome X / FLJ23025 fis, clone LNG01702	71bp	GGAGACAAAATACAGTGGCATTAC TGGAAGGAATATCACAACATTACAT TTTTATCTTAAAGGTCAAGCTT	Induced in Individual 10, but not in Individual 9	AL355476
165						
162	G + AP52	clone RP11-517O1 on chromosome X / FLJ23025 fis, clone LNG01702	71bp	GGAGACAAAATACAGTGGCATTAC TGGAAGGAATATCACAACATTACAT TTTTATCTTAAAGGTCAAGCTT	Induced in Individual 10, but not in Individual 9	AL355476
167						
163	G + AP52	clone RP11-517O1 on chromosome X / FLJ23025 fis, clone LNG01702	71bp	GGAGACAAAATACAGTGGCATTAC TGGAAGGAATATCACAACATTACAT TTTTATCTTAAAGGTCAAGCTT	Induced in Individual 10, but not in Individual 9	AL355476
169						
164	C + AP48	No significant match to anything	103bp	CAAATTGTATTCTTTTAATGAATTTT AATATTCTCAACTAGTATCAATGCT TTGTCATTATTAAGTGTTGACTTCA ATATTTCCCTCCCCTCACCGCAAG CTT	Repressed in Individual 8, but not in Individual 9	
165	C + AP45	dynamitin (dynactin complex 50 kD subunit) (DCTN-50)	61bp	CAGAGTACAACAGCATTTAATGGTC AGAAACAGTTGTACAGTATTACAGT CAGCCAAGCTT	Induced in Individual 10, but not in Individual 9	NM_006400

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Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
166	A + AP3	cyclin D2 (CCND2)	265bp	AGGTCAAGGTGAGTTTATTGTCCAA ATAGCATAACCTAATTGCATTCAAA ACCATTTTCAAATCCATCTTTAAAC TAGTCAGAAAACAGGTTATTATTTT TTTAAATCACTTAACACTGAACAGA TAAGACCTCTTAAAAGGCAGCTGA CTATATCATGTCACCATCATAGCCA ATACAACATTTTTGCCATACTTCCT AAAAACCTTTTCGCATACACTGATC ATGCTACTTATCAACACTTTCTAAC ATCCTGACCAAAGCTT	Induced in Individual 11, but not in Individual 12	NM_001759
167	C + AP24	chromosome 16 clone RP11-296I10	246bp	CCATACATAGGCTTGAACAGGGTT CAGCCAACTTCTTCTGCAGAGAGC CAGAAAGTAACAGTTCTCAGGGAA CTGTAGTCACTGTTGCAGTTACTCA ACTTTGCCATCCTAGTGTGAAAAGC AGCCACTGGCAATACATAAATAAAT GGTGTGGCTGTTTCAATAAAATTTT ATGTATAAAAACAGGTAGTAGACTG GATTTGGCCTAAGTGTTGTAGTTTT TTGATCTATGGGCTAGTGAAGCTT	Induced in Individual 5, but not in Individual 12	AC009060
168	C + AP24	No significant match to anything	253bp	CCAGATTTAATCAGAAAATTGTATC TACAATAATTGACACAACTCACTTC TTGTCCTTCACCCATCTCATCAAAA ATTATTGCATTTTCCCCCAAACTGT ATTTTTTGGATTCCTCTGTGAATAT ACTCATTTCGTCCTCTTTCCTGGAG GCATCTTGGTTTAAATCCATCTTTA AAAAAAGGAGAAGGGGTACATATG TGGAGGCAAAATATTCTCTTGAAGC CAACAGATACAGAGTTGCTAGTGA AGCTT	Repressed in Individual 11, but not in Individual 12	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
168	C + AP24	NADH dehydrogenase	254bp	CATTTTATTTTTATTAAGCACTACAT AACACATTGCATGCTACATAATACA CTGTAACCTTCTAGCAGGGGTAGA TGGCCATAACTGAGTTATTTTTTTC ATCAATCAGGAAAATGCTTCCTTAA TCAATGTTCTCCAAACCCTTTGACA CATAGTAACGATCAACTCCAGAGAT GCGCCTATCTCTTTCCATCAGACTC CAGTGATACCCAAAATGAGCAACC CTTTTTTCCTTGCCCCCGCTAGTGA AGCTT	Repressed in Individual 11, but not in Individual 12	X81900
169	G + AP5	alpha-L-fucosidase	153bp	AAGCTTAGTAGGCTGCTATGGTCA ACTCTCCAGAAATTTTCAGAGCAAT CTAAAAGCGCCAAAATTCGCTATGT TTACAGTGATACTATTAAGAAAATG AATGTGATTCTGCTCTGTCTTTTTA AGTATGATCAAATAAAAAATTTGTA CATC	Repressed in Individual 5, but not in Individual 12	M29877
169	G + AP5	latexin mRNA	139bp	AAGCTTAGTAGGCCAAATAATCCCA AAGTGTCACTTTATATAAATGTCTT GATTACAGTATAGAACTTTATAGAG TCCATAATACAAAGTATCACTACAT AAAAATGTCTTTAAAACAGTAATAG TGGTATGTATATCC	Repressed in Individual 5, but not in Individual 12	AF282626

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
170	G + AP10	manganese superoxide dismutase (MnSOD/SOD2)	294bp	AAGCTTCCACGTATAAACATAAATT GTATTTCCTGTTTTAATTCCAGGGG AAGTACTGTTTGGGAAAGCTATTAT TAGGTAAATGTTTTACAAATTACTG TTTCTCACTTTCAGTCATACCCTAA TGATCCCAGCAAGATAATGTCCTGT CTTCTAAGATGTGCATCAAGCCTG GTACATACTGAAAACCCTATAAGGT CCTGGATAATTTTTGTTTGATTATT CATTGAAGAAACATTTATTTTCCAA TTGTGTGAAGTTTTTGACTGTTAAT AAAAGAATCTGTCAACCATC	Repressed in Individual 11 and Individual 5, but not in Individual 12	X65965
171	G + AP10	metallothionein-IG (MT1G)	137bp	GGGAATCAAGTCTAAGTGTTTAATT ATTATTCACATATTTCACAGAAAAA AAGGAATGTAGCAAAGGGGTCAAG ATTGTAGCAAAAAACAAAAAATCCT GGATTTTACGGGTCACTCTATTTAT ACGTGGAAGCTT	Induced in Individual 11, but not in Individual 12	J03910
172	A + AP23	cyclin D2 (CCND2)	179bp	AGGTCAAGGTGAGTTTATTGTCCAA ATAGCATAACCTAATTGCATTCAAA ACCATTTTCAAATCCATCTTTAAAC TAGTCAGAAAACAGGTTATTATTTT TTTAAATCACTTAACACTGAACAGA TAAGACCTCTTAAAAGGCAGCTGA CTATATCATGTCACCATCATAGCCA AGCTT	Induced in Individual 11, but not in Individual 12	NM_001759

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Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
173	A + AP3	cyclin D2	265bp	AGGTCAAGGTGAGTTTATTGTCCAA ATAGCATAACCTAATTGCATTCAAA ACCATTTTCAAATCCATCTTTAAAC TAGTCAGAAAACAGGTTATTATTTT TTTAAATCACTTAACACTGAACAGA TAAGACCTCTTAAAAGGCAGCTGA CTATATCATGTCACCATCATAGCCA ATACAACATTTTTGCCATACTTCCT AAAAACCTTTTCGCATACACTGATC ATGCTACTTATCAGCACTTTCTAAC ATCCTGACCAAAGCTT	Induced in Individual 10, repressed in Individual 9	NM_001759
174	A + AP3	clone RP11-120K22 on chromosome 6	182bp	ACATTCTTTCAAAGGGTTCTTTATA GACTGTCCATGTCTTTACAATCAAA TATCTTCTAATTGTATATTACTACCT TGGCTTCTAATTAGTTTCCCCTCCT TAACAAGCAAGGAAATGGAACAGG TAATATCTAAAATTAAAGTAAAACC ATGGAATAGTTCTAGGACTCTGAC CAAAGCTT	Induced in Individual 1, but not in Individual 9	AL137178
175	G + AP41	Familial Cylindromatosis cyld gene	241bp	GGCAAAACATCACAGAATATAACTG TTCCAGATTCTATTGTTTCTCCAAC CTTCAAAGAAACTCTGGAGTTTATT TCCAAAGGAGGAAGTTCGACCTGC ATTGTGTCCCCAGGACCTGCGTAA TCACTTTCCAATGCAGTGTCATCAT CTTCTATGAGTTCTAGCTTGTCCAA TGCAACAAACACGCCACAATCTTCA TCACACTGAAAAAGCTGTTTCCCTT GGTACACCCCGTAAGCTT	Repressed in Individual 11, but not in Individual 12	AJ250014

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
176	G + AP41	ribosomal protein L8 (RPL8)	218bp	GGCATAAACACAAACTTTATTGAGG CCCTCAGCACTAGTTCTCTTTCTCC TGCACAGTCTTGGTTCCCCGGAGA CGTCCAGTCCGGCGGGCAGCAAT GAGACCCACTTTGCGGCCAGCAGG GGCATCTCTGCGGATGGTGGAGG GCTTGCCGATGTGCTGGTGGTTGC CACCTCCAAAAGGATGCTCCACAG GATTCATGGCCACACCCCGTAAGC TT	Induced in Individual 11, but not in Individual 12	NM_000973
177	G + AP42	No significant match to anything	241bp	GATACTAAATGGTTTTTGCCAGCAA AAAGGAGTGAACCTACTTCTTAAAA CACCCGAGTAACCAAGCTGCTACT AAGACTAGAGGGATGCTAATTCTG GCAAAAGTCAAAGGTTACTAACAAA AATTAGCTAAGATTATATGTTTGCA GAAAGTCAATTCAGGGTAATTCCG CTATTATCCTGTTGTGTTTATTATTC CTAAATAACTGGCAATTCCACCTTA AACTACGGTGCAAAGCTT	Repressed in Individual 11, but not in Individual 12	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
178	G + AP42	cDNA FLJ10589 fis, clone NT2RP2004389	330bp	GAAACTAACATTCTTTATTTTCCTTT TATTTTTTAAAGGTCACCTTTACAAA GTAGCATTTAAAAATAAATCCATCT CACAGCTCAAAGAAATTTCTGACAG TACTGCCCTCATGCTGGTCAGCCA TTATTTACTGTGTGTCTGCCACATG TCGGCACATATATATAGCTCTTCCT CTCCTTTCCTGGGAGCAAACCCTT AGCGTTTCTTCCACGTAAACTTTCT GCGGGCTCCCTCTTGGCCTGGCTT CTTCCGTTCCCTCACACGTGGATC AGTAGTAAGTAGTCCAGCTTGTCTC ATCCACTCGACCTCGTCCTCGGTG CAAAGCTT	Repressed in Individual 11, but not in Individual 12	AK001451
179	G + AP47	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein) (EEF1D)	217bp	GCCGGTCTCAGTCTTTAATCGTGG CAGGGCCTCACGCACGCGCGCAC GTACACACACTCAGGCTTCAGATC TTGTTGAAAGCTGCGATATCGACA CTCTGCACGTGCTCCTCAAACTTG GTGATCTCCTCCTCCAGCAAGTCT GTCCCCACCTTGTCGTCCTCCACC ACACACTGAATCTGTAGCTTCCGG ATACCGTAGCCCACGGGCATAAGC TT	Repressed in Individual 11, but not in Individual 12	NM_001960

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
180	G + AP47	Chromosome 16 BAC clone CIT987SK-A-67A1	270bp	GATTCAGGGTCTCGTTATGTCACCT AAGCTACAGTGCAATGGCACAATC ATAGCTCAACACAACCCTGAGTTTC TGGGCTCAAAGGATCCTTCCACTT CAGTCTCTAGAGTAGCTAGGACAT CAGGAGTGTGCCACCAAACCTAGC TTTTTTTTTTTTTTTTTANATATAGA AGTCCCACTAGGTTGCCCTGGCTG GNCTTAAACTCTTGGTTTCAAGTGA TCCTCCTGCCTTGGCCTTCCAAAAT GCTGGTATTACGGGCATAAGCTT	Induced in Individual 5, but not in Individual 12	AC004531
181		No insert - just vector				
182		No insert - just vector				
183	A + AP41	proteasome (prosome, macropain) subunit, beta type, 8 (large multifunctional protease 7) (PSMB8)	451bp	AGCTTACGGGGTCATGGACAGTGG CTATCGGCCTAATCTTAGCCCTGAA GAGGCCTATGACCTTGGCCGCAGG GCTATTGCTTATGCCACTCACAGA GACAGCTATTCTGGAGGCGTTGTC AATATGTACCACATGAAGGAAGAT GGTTGGGTGAAAGTAGAAAGTACA GATGTCAGTGACCTGCTGCACCAG TACCGGGAAGCCAATCAATAATGG TGGTGGTGGCAGCTGGGCAGGTC TCCTCTGGGAGGTCTTGGCCGACT CAGGGACCTAAGCCACGTTAAGTC CAAGGAGAAGAAGAGGCCTAGCCT GAGCCAAAGAGAGAGTACGGGCTC AGCAGCCAGAGGAGGCCGGTGAA GTGCATCTTCTGCGTGTTCTCTATT TGAACAAGCATTTCCCCCAGGGAA GTTTCTGGGTGCCCCACTAAGTAG AATAAAGAAAAACGGTTAT	Expression is higher in Individual 5 and Individual 11 then in Individual 12	NM_004159

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
184	G + AP42	lectin, galactoside-binding, soluble, 9 (galectin 9)	153bp	AAGCTTTGCACCGTGCACCAACCC TTCACCCCTCCTGGAAAGCAGGCC TGATGGCTTCCCACTGGCCTCCAC CACCTGACCAGAGTGTTCTCTTCA GAGGACTGGCTCCTTTCCCAGTGT CCTTAAAATAAAGAAATGAAAATGC TTGTTGGC	Repressed in Individual 5 and Individual 11, but not in Individual 12	NM_002308
184	G + AP42	polymerase (RNA) II (DNA directed) polypeptide B (140kD)	201bp	GACTATCTACAAAAATTTATTATATA TTTACAGAAGAAAAGCATGCATATC ATTAAACAAATAAAATACTTTTTATC ACAACACAGTACATATTTGTCATTT TTAAAAAGCCACACAATAGAAACAA GACACCAAGATATTTAATTATCTTG TTGACTCCTGTAAAATAGCTAAACA CTCATCATTCGCGGTGCAAAGCTT	Repressed in Individual 5 and Individual 11, but not in Individual 12	NM_000938
185	A + AP47	Chromosome 16 BAC clone CIT987SK-A-67A1	106bp	AGATATAGAAGTCCCACTAGGTTG CCCTGGCTGGTCTTAAACTCTTGG TTTCAAGTGATCCTCCTGCCTTGG CCTTCCAAAATGCTGGTATTACGG GCATAAGCTT	Induced in Individual 11, but not in Individual 12	AC004531
186	A + AP46	UbA52 gene coding for ubiquitin-52 amino acid fusion protein	103bp	ACACGACTGAGGTTTTTACTCCAGT TTTACAGATGACAAATCCAGAGTCA GGGGCATAGGGCAGGGACCAATG CCGATGTGACAGAATCTAGGACCG AAGCTT	Slightly induced in Individual 11, but not in Individual 12	X56997

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
187	A + AP44	small nuclear ribonucleoprotein 70kD polypeptide (RNP antigen) (SNRP70)	206bp	AAGCTTCTCCGGAGAATGGGTATT TGATGGAGGCTGCGCCGGAGTGA AGAGGTCGTCCTCTCCATCTGCTG TGTTTGGACGCGTTCCTGCCCAGC CCCTTGCTGTCATCCCCTCCCCCA ACCTTGGCCACTTGAGTTTGTCCT CCAAGGGTAGGTGTCTCATTTGTT CTGGCCCCTTGGATTTAAAAATAAA ATTAATTTCCTGTT	Induced in Individual 11, but not in Individual 12	NM_003089
187	A + AP44	isocitrate dehydrogenase 3 (NAD+) gamma (IDH3G)	204bp	AAGCTTCTCCGGACATCGGGGGCC AGGGCACAACATCTGAAGCCATCC AGGACGTCATCCGCCACATCCGCG TCATCAACGGCCGGGCCGTGGAG GCCTAGGCTGGCCCTAGGACCTTC TTGGTTTGCTCCTTGGATTCCCCTT CCCACTCCAGCACCCCAGCCAGCC TGGTACGCAGATCCCAGAATAAAG CACCTTCTCCCT	Induced in Individual 11, but not in Individual 12	NM_004135
188	A + AP44	no insert - just vector				
189		clone RP4-646P11 on chromosome 1	238bp	AAGCTTTGAGACTAGCCTGGGCAA CATGGAGAAACCCCATCTCCACTA AAAATACAAAAAGTGGATGGGCAT CTTGGCGGGCAACTGTAATCACCA CTAATCGGGAGGCTGAGGCAGAAG AATCCTTTGAACCCAGCAGGCAGA TGTTGCAGTGAGCCAAGATTGCAC TATTGCACTCCAGCATGGGTGACG GGGCAAGACTCGTCAAAAAACAAA CAAACAAAACGATAAATAAATC	Repressed in Individual 11, but not in Individual 13	AL049715

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
190	A + AP52	hypothetical protein FLJ20436 (FLJ20436)	204bp	AGGGGAGTGTGCTAGTACAGAGGA ATCCTTTTATGATCAACTAGAGTCT GGGTCCTTCACTTTCAGTCGAGTG CTGTCTCCATTAGACCACAGTAAAG AGTTAACAGGTCACCAATGGCAGT CGCATCTTTCTGGGTAAGGCAGTG TCTGGTCATTGGAAGAGACTGATT GGAACAACGAACATCATCCACAAA GGTCAAGCTT	Induced in Individual 11, but not in Individual 13 or Individual 5	NM_017822
191	G + AP49	c-Cbl-interacting protein (CIN85)	317bp	AAGCTTTAGTCCATAGTGGTACTAT TTTGATGATATTTTCCATTAATAAAA TGTAATTTCAGATTATTCGTTTACAA GCTTTATAATTTTATGATTTTTTAAT CGTGTTTTGTCACAGACTTCCCTAG TGTTTGTACTACACGTAGTCAGAAG CGAGTGTCCTTTTCTTTTGCTTCAG GCTAAGAGCTGCCTCGCTCTTTGT CCCCCCATTATGATTCTATTACATA TGCAATTGTAGGTTCAACCTGTCCC TTTCCCTGCCAGCAAACCCCACCA CCCTAAGAGAAATTTTAGCTTATAT ATGACGGTATATTTAC	Repressed in Individual 11 and Individual 5, but not in Individual 13	AF230904

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
192	G + AP49	ribosomal protein L7a (RPL7A)	293bp	GGCTGAAGGAAAATTTGTATTATTT CAATTATTTTTATGTACAGAAAACT CAACAGTGTACATTTAACCCAGTTT AGTGGCAAGTTCTTTAGCCTTTGCC TTTTCGAGCTTGGCGATACGAGCC ACAGACTTAGGACCCAGGACATTG CCACCCCAGTGACGGCGGATCTCA TCGTATCTGTCATTGTAATTGGTCC TGATAGCTTCCACCAGCTTAGCCA AAGCGCCTTTGTCTTCCGAGTTCA CCTGTGTGAAGGCGACAGTGGTGC AGGTCTTCCTGTGGACTAAAGCTT	Induced in Individual 11, but not in Individual 13	NM_000972
193	G + AP51	ribosomal protein S21 (RPS21)	318bp	GGTAGGTTTTCATTATTTATTTATGA CAAATATTCCACATCTGTGATTCTC TCCAGTCAAAAGTTCTTTGAGACGA TGCCATCGGCCTTGGCCAATCGGA GAATGGAATCATCTGACTCACCCAT CCTACGAATGGCCCCGCAGATAGC ATAAGTTTTAAACTGGCCATTAAAC CTGCCTGTGACCTTGTCAACCTCG GCCACGTTCATCTGGATGGATGCG TGGTCCTTGGCACCGATGATGCGA TTGCTAGCGGAGCATTTCCGCGGC ACGTACAGGTCCACGAACTCGCCG GCGTCGTTCTGCATTTCGAAGCTT	Induced in Individual 11, but not in Individual 13	NM_001024

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
194	G + AP50	Chromosome 16 BAC clone CIT987SK-A-67A1	150bp	AAGCTTTGAGACTTGGAAAGGTAC GCTGCTGGCCAGCATTGGAGAAGA AGCTGCTGAGCATGGCTTTCTGTA GTCTTTAGCAAGACACAAGTGGATT TTGACTTTGTATCATGTCATGATTT CTAACAATAAATGATGTTTTTATGT GCC	Induced in Individual 11, but not in Individual 13	AC004531
195	G + AP50	sorting nexin 6 (SNX6)	132bp	AAGCTTTGAGACTAAACCAAGTATT GTAAAATAAACAGCGATAACAGTGA TAGTTTTTAACTCTATGGTCATTGT ATCACTCTGGAAAATGTGGAGTAG CTGTAATAAATCTACTCCTGTATTA TGCTTTAC	Repressed in Individual 11, but not in Individual 13	AF121856
195	G + AP50	No significant match to anything	133bp	AAGCTTTGAGACTATGTACAAATAC ACTAAAGTGGTGATGGTGATCAATA TTGTNAAGAATTTATTCTGATAAAT GAGAAACTGGATATAATGTCAAAAT AAGCTATTTTCTCAATAAAAATCTC AAATCTCC	Repressed in Individual 11, but not in Individual 13	
196	G + AP50	TNF-inducible protein CG12-1 (CG12-1)	87bp	GGTGAGTAGGTGAGTTTATTAGGA CTTACACACAGGGCACTCAGCAGG ATGGCTCTAGAGATCCGGCCTCCC CCAGTCTCAAAGCTT	Repressed in Individual 11, but not in Individual 13	NM_014349
197	G + AP50	BRCA2 gene region chromosome 13q12-13 contains xs7 mRNA	55bp	GTATTTTTAGTAGAGATGGGGTTTC ATCATGTCTGCCAGGCTAGTCTCA AAGCTT	Repressed in Individual 11, but not in Individual 13	Z75887 Z75888
198	G + AP51	ribosomal protein S24 (RPS24a)	160bp	GAAGTTTTTAGTTTATTAATGTTCTT GCGAAAAATCCACAGTGGCCACAG CTAACATCATTGCAGCACCTTTACT CCTTCGGCTTTTTGCCAGCACCAA CATTGGCCTTTGCAGTCCCCCTGA CTTTCTTCATTCTGTTCTTGCGTTC ATTTCGAAGCTT	Repressed in Individual 5 and Individual 11, but not in Individual 13	NM_001026

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
199	G + AP51	No significant match to anything	120bp	GGCATCTTGGGTTACTATACTCACT CATGCAAAATCCAAGGTAGTGTAAT GGAAACAATATTGGTAAAATAGATG TTGTGGCTATAAGCATCTCCCTTTA TATTAGACATTTCGAAGCTT	Repressed in Individual 11, but not in Individual 13	
200	G + AP52	CGI-128 protein mRNA	146bp	GGGAACTGAATATAAATTAACTTTA TTACAAAAAGCAAAATGTTAGTTTC TCATTGTGAGTGATTCAAGAAAACA ACGGTAACAGCCCTGGCAGGAGCT GGGACCAGGATACCAGTATGCAGG CTGAGGGGTCAAAGGTCAAGCTT	Induced in Individual 11, but not in Individual 13	AF151886
201	G + AP41	ribosomal protein L8 (RPL8)	218bp	AAGCTTACGGGGTGTGGCCATGAA TCCTGTGGAGCATCCTTTTGGAGG TGGCAACCACCAGCACATCGGCAA GCCCTCCACCATCCGCAGAGATGC CCCTGCTGGCCGCAAAGTGGGTCT CATTGCTGCCCGCCGGACTGGACG TCTCCGGGGAACCAAGACTGTGCA GGAGAAAGAGAACTAGTGCTGAGG GCCTCAATAAAGTTTGTGTTTATGC C	Induced in Individual 11, but not in Individual 13	NM_000973
202	G + AP41	Familial Cylindromatosis cyld gene	241bp	GGCAAAACATCACAGAATATAACTG TTCCAGATTCTATTGTTTCTCCAAC CTTCAAAGAAACTCTGGAGTTTATT TCCAAAGGAGGAAGTTCGACCTGC ATTGTGTCCCCAGGACCTGCGTAA TCACTTTCCAATGCAGTGTCATCAT CTTCTATGAGTTCTAGCTTGTCCAA TGCAACAAACACGCCACAATCTTCA TCACACTGAAAAAGCTGTTTCCCTT GGTACACCCCGTAAGCTT	Repressed in Individual 11, but not in Individual 13	AJ250014

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
203	G + AP47	Tu translation elongation factor, mitochondrial (TUFM)	358bp	GGCTTACTATTCAAAGTTTACTGAC CTCCCCAGCCAGGCAGGCCAACC CTTCCGAGCAGGGGAAATGTCCAT CTAGCTGCCCTCTGCTGGGTTGCA GCCTATGCCATGAGAGGGTACTGG AAGCAGGAGGGAGCCCTGGCTAG GGCAGGCCTTAAACGCAAGGGAAG CTGAGCAGAGATCTGCACACTCAA CCCCATTTGATATTCTTCTCCTCCT CAGTCATGGCCAGCGTGTTGGTGA CTAGACCGGTGCCAATAGTCCGGT TGCCATCTCGCAGGGTGAAACGCT GGCCTTTCTCTAAGATCATTGGCTG CCGCAAGATTAGGTTGAACTTCAN GTCCTCCCCGGGCATAAGCTT	Induced in Individual 11, but not in Individual 13	NM_003321
204	G + AP47	KIAA0787 protein	200bp	AAGCTTATGCCCGATGCTTCTGTTT CATTCCCGACCCTTTCTACTATGCA TTTTCCTTTTATCAGGTGTATAAAG TTAAATACTGTGTATTTATCACTAAA AAGTACATGAACTTAAGAGACAACT AAGCCTTTCGTGTTTTTCCACAGGT GTTTAAGCTTCTCTGTACAGTTGAA ATAAACAGACAGCAAAATGGTGCC	Induced in Individual 11, but not in Individual 13	AB018330
204	G + AP47	ribosomal protein L13 (RPL13)	203bp	GATTCCAAGTCCCCAGGAGGGCTT TATTTTTTCTTTTCAACATCCTGTTC TGCGGCTTCCTTGGCTCTTTTTGC CCGTATGCCGAAGAGCCGGGCGTT GGCACGGGCCATACGGAGACTAG CGAAGGCTTTGAAATTCTTCTCTTC CTCAGTGATGACTCGAGCTTTCTC CTTCTTATAGACGTTCCGGACGGG CATAAGCTT	Induced in Individual 11, but not in Individual 13	NM_000977

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
204	G + AP47	fatty acid synthase/ NY-REN-57 antigen RNA	202bp	GGAGATCACATGCGGTTTAATTGT GGGAGGCTGAGAGCAGCATTTTTA CCAAATTGGTGTAAAAATGAAACG GGGTCCAGACGTGTACACTGACAG TTACAGTAAATTTCAAAATCCAAGC AGCAATTTGAATCATTTCTTGAAAA ACAAACACAGACAAACACCAAACAT GGAGTTGGTGCCCGGCGCCGGGC ATAAGCTT	Induced in Individual 11, but not in Individual 13	S80437
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204	G + AP47	ribosomal protein L19 (RPL19)	198bp	AAGCTTATGCCCGCAGGTCTAAGA CCAAGGAAGCACGCAAGCGCCGT GAAGAGCGCCTCCAGGCCAAGAA GGAGGAGATCATCAAGACTTTATC CAAGGAGGAAGAGACCAAGAAATA AAACCTCCCACTTTGTCTGTACATA CTGGCCTCTGTGATTACATAGATCA GCCATTAAAATAAAACAAGCCTTAA TCTGC	Induced in Individual 11, but not in Individual 13	NM_000981
205	G + AP45	clone 245M18 on chromosome 6p21.32-22.3	186bp	GGAAGGAATATTCTAAATGATAAGA TATGCTCTGGCTACACAGCACAAA ATAAAGATGGTTTTTAGCTTACATG ATCTGATTTGTCTGTCAACAAGAAT CCAACAGTTCAGGCTCTTGACACG TCCCTCTTCAGCCTAGGGTGTGCA GATGTTATGGAATCATCACCCTCAT AGTCAGCCAAGCTT	Repressed in Individual 11, but not in Individual 13	AL078584

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
205	G + AP45	clone TCBA00781	184bp	GACTGTCCTAAATTGTTTATTAAGT ATGAATTTTACAAACTTTACTTATAT TAGCGGTAACGGTGGAGCTGGAGA GTATTGCGCCTTCTCCAAGCTGCC CGGCGAGAGCCACCAATAGTGTGG TGGAACTTGTGGCCCTTTCCAAGG CCACGGCTCTTTCGGCCTGCAGAT GTCAGCCAAGCTT	Repressed in Individual 11, but not in Individual 13	AF283772
206	G + AP45	chromosome 19, cosmid R26529	198bp	AAGCTTGGCTGACGTTATGGTGGC TTCAGCTTCACTAGGAATGGGACA CAGGGTCTGGGGGCCTCTGACTCC CCCACCCCGAGGCCTGGGTAGGG ACAGGGTGGTGGTCCCTGGGTGG GTCAGGTAGGGCACAGGGGCCAG GGAGGGACAAGCAGACCTCAGAG CGCTGCCCAGATGGAATATTAAATT ATTTTTGCC	Induced in Individual 11, but not in Individual 13	AC005551
207	G + AP47	tumor suppressing subtransferable candidate 1 (TSSC1)	182bp	GCAGCGTGGATTTATTTTATTTCAT TTTTTACTCTCAAGAGAAAGAAGAG TTACTACTGCAGGAACAGACATTTT TTTAAAAAGCGAAACTCCTGACACC CTTAAAACAGAAAACATTGTTATTC ACATAATAATGTGGGGCTCTGTCTC TGCCGACAGGGGCTGGGTTCGGG CATAAGCTT	Induced in Individual 11, but not in Individual 13	NM_003310
208	G + AP43	glyceraldehyde-3-phosphate dehydrogenase (GAPD)	74bp	GGTTGAGCACAGGGTACTTTATTG ATGGTACATGACAAGGTGCGGCTC CCTAGGCCCCTCCCCGCTTCAAGC TT	Induced in Individual 11, but not in Individual 13	AF261085
209		no insert - just vector				

Band	Primer Combination	Identification	Size	Sequence	Expression In Individuals Tested	GenBank Accession number
210	G + AP52	transferrin receptor (TFRC)	105bp	GCACATAACAGCTTTTATACAATGA TAAGGACATATCATTTGTTTACAAA GAAAGTCTAAAATTTCAAGAACATT CAAAGAGCTAACACAGTAAAGGTC AAGCTT	Induced in Individual 11, but not in Individual 13	AF187320
211	G + AP55	Homo sapiens PAC clone RP4-649M7 from Xq23	287bp	GTAAATTTGTTTGAGTTCATTGTAG ATTCTGGATATTAGCCCTTTGTCAG ATGAGTAGGTTGTGAAAATTTTCTC CCATTTTGTAGGTTGCCTGTTCACT CTGATGGTAGTTTCTTTTGCTGTGC AGAAGATCTTTAGTTTAATTAGATC TCATTTGTCAATTTTGTCTTTTATTG CCGTTGCCTTTGGTGTTTTGGACAT GAAGTCCTTGCCCATGCCTATGGC CTGAATGGTAATGCTTAGGTTTTCT TCTAGGGTTTTTATGGTTTTATGTC TAACGTAAGCTT	Induced in Individual 11 and Individual 5, but not in Individual 13	AC006968
212	G + AP54	ubiquitin-conjugating enzyme E2D 3 (UBE2D3)	142bp	AAGCTTTTGAGGTTACATGATATGC TTTATGCTCATAACTGATGTGGCTG GAGAATTGGTATTGAATTTATAGCA TCAGCAGAACAGAAAATGTGATGT ATTTTATGCATGTCAATAAAGGAAT GACCTGTTCTTGTTCTAC	Induced in Individual 11, but not in Individual 13	AF224669
212	G + AP54	putative DNA-directed RNA polymerase III C11 subunit	142bp	AAGCTTTTGAGGTGAAGAGCCAGG GGGTCAGGAAATATGGCCTATCTG CCAGGCAGGGTGGATGAAGTCATG AATGTCTGGGAGTTTTTCTGTGTGG GGAGGAGACAGAGACCCATAACTA AATATGCTCTGTGTAAAGTCC	Induced in Individual 11, but not in Individual 13	AF126531
213	G + AP54	testis-specific kinase 2 (TESK2)	89bp	AAGCTTTTGAGGTACAAGTAAGAA GGCTGACCAGCACCTGTAACACTG ACTTTATTTTTAAGTCTGAAAATGTC TTGGGAAAGTTTTAC	Induced in Individual 11 and Individual 5, but not in Individual 13	NM_007170

Band	Primer Combination	Identification	Size	Sequence	Expression In Individuals Tested	GenBank Accession number
213	G + AP54	integrin, beta 2 (antigen CD18 (p95)	87bp	GTAAATAAATTGGCACCACCTTTAA TCAGACTGATGTCCTGACTTGCAC AGGAAACACGCACCTAACCTCACC AACCTCAAAAGCTT	Induced in Individual 11 and Individual 5, but not in Individual 13	NM_000211
214	A + AP49	No significant match to anything	111bp	AAGCTTTAGTCCACTTTACAACAAA GAGCAGCTTGTCTTTGCAGCTTTGT TAGCTCTTAAACTTCCAGATTAACT GTGTAGCCATTTCAGTAGCACTAAA AGATTAACTCT	Induced in Individual 11 and Individual 5, but not in Individual 13	
215	G + AP49	PAC clone RP4-726N20 from 7q32-q34	139bp	AAGCTTTAGTCCAATTAGGGAGTAA AAGGAGGGAAGGGGCCTATCCATT CCATTGTGGAAGCTGGGCCAGGTG CCAGGGACACTCTCCTTCAGGGAA AATGTTATGTGGAGGAGGACGAAT AAATTTATTTTGTTTTCC	Induced in Individual 11, but not in Individual 13	AC006344
216	G + AP49	BAC R-487K10 of library RPCI-11 from chromosome 14	133bp	GGGGAGAAAATGTATCAAAGGAGT TTATTAAAAGCAATGGTAGTATCAG TCCTAAAGCAAACACTGGAACAAAA TAAAAACACAAAAACCTTTTCTCGG ATATGAAATCATCGATTATATTGGA CTAAAGCTT	Induced in Individual 11, but not in Individual 13	AL352976
217	G + AP49	F-box protein FBL4 mRNA	100bp	AAGCTTTAGTCCATAATATGATTGA TAAAAGAATAACATGGAATCATGCT AACTTATTTTCAAAGGAACACTGAG CAATAAAGTATCGTGGCATTTATGC	Repressed in Individual 11, not in Individual 13	AF176699

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
218	G + AP11	cDNA DKFZp762H1311	215bp	AAGCTTCGGGTAAGGATGAGGAAC TCTGAAATCAGATGGAAAAGAGCG GTGTTAATTTTTATGGTCTGTGATC GTAGCTGTGATAAGGGACTGAGGA ATAAATTGTGCTCTTTGTCATGGCA ACCAGCTTCTGAAAAGCCCACTGA AAATTGCCTGTCCTGCTGGTAACT GCTACGGGGTAAGATTTGCCTTAA CAGTACTATTTTCTCGCCACC	Repressed in Individual 5, not in Individual 13	AL359617
219	G + AP4	cDNA DKFZp434F2021	63bp	GGCTTCAACTACATGTCAGGTGTTA ATATGAAAAATCTGCCACAGCCTAC CGTTGAGAAGCTT	Repressed in Individual 5, not in Individual 13	AL117573
220						
221	G + AP19	No significant match to anything	233bp	GGTATGTTTTTAGCAGCACTCTTTT TTCATCAAATCGGATTATTTTAATTT TTAGATTTAAATTGTGTTAATAAAAA AGCCCTCATTCATGAGGGCTTTATT TTATGGATGATAAATTGCTTAACCG TGACGTTTTGCAAACTCGTCCATGA AATTAACCAATGCTTGTACCCCTTC TAAAGGCACAGCATTGTAAATACTT GCACGCATACCACCCACAGAGCGA TAAGCTT	Repressed in Individual 5, not in Individual 13	
221	G + AP19	neural precursor cell expressed, developmentally down-regulated 8 (NEDD8)	239bp	AAGCTTATCGCTCATAATGAGGCAT CATATATCCTCTCACTCTCTGGGAC ACCATAGCCACTGCCCCCTCCCCT GGATGCCCAGTAATGTATGTCTACT GGTGGGAGACTGTGAGGATCCCA GGATTCAGTATTCCTGGCCCAGAG GGCCCTTGCTGGCTACTGGGTGTT AGTTTGCAGTCCTGTGTGCTTCCC TCTCTTATGACTGTGTCCCTGGTTG TCAATAAAATATTTCCTGGC	Repressed in Individual 5, not in Individual 13	NM_006156

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
222						
223		BAC C-2149C7	276bp	AAGCTTCACTAGCTAGACTAATAAA GAAGAAAAGANAAAAGATCCAAATA AACACAATTAGGAATGACAAANGG GATATTACCGNTGACGCCACAAAA ATACAAATAACCATCACAGACTATG AACACCTCTATGCACAGAAACTAGA AATTCTAGAAGAAATGGATAAATTC CTGTACAAATNCATCCTCCCATACT GAACCAGGAGGAAATTGGATNCNT GAACAGACCAATAATGAGCTCTNA AATGGAATCAGTNATNAATAGCCTA CTACC	Induced in Individual 5, but not in Individual 13	AL132827
224		Rattus norvegicus mitochondrial cytochrome c oxidase subunits I, II and III, and ATPase subunit 6 genes	173bp	AGGATCCTCATCAATAGATAGAAAC GTATAGGAATAGTCAAACTACATCT ACGAAGTGTCAGTATCATGCTGCG GCTTCAAATCCGAAATGATGTTTTG ATGTGAAGTGGAATTTTAGTTGTCG TAGTAGACAGACAATTAGGAAAGTT GAGCCAATAATTACGTGGAAGCTT	Slightly repressed in Individual 11, but not present in anyone else	M27315
225	A + AP10	cytochrome c oxidase subunit III gene	116bp	AGGCGGCGGCTTCGAAGCCAAAGT GATGTTTGGATGTAAAGTGAAATAT TAGTTGGCGGATGAAGCAGATAGT GAGGAAAGTTGAGCCAATAATGAC NTGAAGTACGTGGAAGCTT	Induced in Individual 11 and Individual 5, but not in Individual 13	AF004341
226		No insert - just vector				

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
227		No significant match to anything	322bp	GAGGTTTCAACTTAACATTTATTGC ACAACTCACTTCAGGCCACTGCTG ACATCCCAAAACACAGCCTAGTAA CACACAACTTCACCTTCAAGGACT GCAGAGATAGACCAACTATATATAA ATAATCTCGTGGAAACGTAATTTGG GTGAGAAAAATATGACGCTAGAGA CTTTGCGATGAATATTTTGCCTAAG TTCAGAAGCTTGAAAATACAGTTGG CCTACAATCTCAAATTTCACCCAAT AACCGTGGCCCTTAGTCACCACAC CTTCCATCATCTTTTTACCTCTGCC TGTTCTTGCCCTTTACAGTCGAAGC TT	Induced in Individual 10, but not in Individual 9	
228		No significant match to anything	136bp	AAGCTTCGACTGTACATCATGAATA AATCCTAGGGATCTACTGTATACTA TAGTGACTATAGTTAGTGATACTGT ATAGCATACTGGAAATTTTTCTGAG AGGGTAAATTTTACATTAAGTGTTT TTATCTGCCCC	Repressed in Individual 10, but not in Individual 9	
229		cDNA YI27F12	127bp	AAGCTTCGACTGTAGTACCCTTTTC ATGGCATTTACTTACCATAGCCTAT TTGTTATTGCCCTCAAGCTCCATTA GGGCAAATTGTTTTTTCATTGTTTT AAAGTCAGCACCTAAATCTCCCTG GCC	Repressed in Individual 10, but not in Individual 9	AF075018
230	G + AP2	ribosomal protein L8 (RPL8)	66bp	GGCATAAACACAAACTTTATTGAGG CCCTCAGCACTAGTTCTCTTTCTCC TGCACAGTCGAAGCTT	Induced in Individual 10, but not in Individual 9	NM_000973

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
231	G + AP4	CGI-51 protein mRNA	371bp	GTGTCTTTAACATTTATTGACGGGG TTTCCCACAGGGTCCGCAGTCAAA GAATCGCTNGAACCGCGTTTCCTC GAGAGACGGTGTGTGGCATGGGC GCCTTGCTGCTGCCCCAGTCCCAG AGCTTCTCCTGTAGGGGTGCCGGC TACAGGAACCTTATCCCAGCTCCA AACTGGACGCCATCACATATCCTG TCACCTGTCTGTACTCCCATGGGG ACGCAGTAATTAAGTTCCAACCGA GCGATGTTGCCAAGCCTGAGGACA ATCCCGGCCCCGTACGACCAGCG GATGCACTCAGCCAGCTTACGAAT ATGAGCTTTGGGGCCCTCCCCATA GTTGAGGTTGCAGAGGTTTCCTGC GTTGAGAAGCTT	Induced in Individual 10, but not in Individual 9	AF151809
232		BAC clone RP11-357D2 from 7q21.1-q21.2 or cosmid U107D4	228bp	GCATAGGATTGACTTGGCAATGCG GGCTCTTTTTTGGTTCCATATGAAC TTTAAAGTAGTTTTTTCCAATTCTG GGAAGAAAGTCATTGGTAGCTTGA TAGGGATGGCATTGAATCTATAAAT TACCTTGGGCAGTATGGCCATTTTC ATGATATTGATTCTTCCTATCCATG AGCATGGAATGTTTTTCCATTTGTT TGTATCCTCTTTTATTTCGTTGAGA AGCTT	Repressed in Individual 10, but not in Individual 9	AC006374

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
233	G + AP4	ALL1-fused gene from chromosome 1q (AF1Q)	212bp	GGGATTGAATGTCTTTATTAAATAA ACGAGTAAATGGTAGCACAAATCA CCATCAATATTTTTGGAAGGATTGG GGACAAGATGTCGAGTCAGAATAT AATTGTTCATTTCAGGGTCTCAATG TAGCTGAAGAACTGTGCCCACTGA TCAGTATTACGTATTGCAAATGCAG GAGGTAAGGCTAAAATAGGACTTA TGCCGTTGAGAAGCTT	Induced in Individual 10, but not in Individual 9	NM_006818
234	G + AP9	BAC R-11K13 of library RPCI-11 from chromosome 14	365bp	AAGCTTCATTCCGGTACTCAATTGC TCCCTAAGAGTATCAATATTCTTGG CATCACAACAGATACAGTATTCTAT TGTTTTTTTTTCAACTCAATTGTAAA ACTTTGGAGAGAAGGAACCATCAA TTTTTGCAGATATACAAACAGAGAT ATTCCACAAAGACTAATAGAAAGCA AGACTCACATGCACATACCTATGTA TAAAAGAACACCTACATAAATTAGA ATAAGGAAGGAGAGGCCGGGCGT GGTGGCTCACACCTGTAATCCCAA CACTTTGGGAGTCCGAGGAAGGTG GATCACCTGAAGTCAGGAGATCGA GACCATCCTGGCCAACGTGGTGAA ACCCTGTCTTCACTAAAATAC	Repressed in Individual 10, but not in Individual 9	AL355095
235		Insert is too small				
236	G + AP12	BAC R-307P22 of library RPCI-11 from chromosome 14	76bp	AAGCTTGAGTGCTGCTAGGCCAGA AAATTACATTTAGCATCCTTCTGGT AAATTACATTTAGTTAGTGTCCTTC CC	Repressed in Individual 10, but not in Individual 9	AL132777

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
237		clone RP3-505P2 on chromosome 6	281bp	AAGCTTCACTAGCTAGAGTAACAAA GAAAACGAGAGAGACAAAAGATCC AAATAAGCACAATGAGAAATGACAA AGGCAATATTGCAACCAATACCACA GAAATACAAAAGGTCCTCAGAGAC TATTATCAACACCTCTATGTGCACA AACTAGAAAATCTAGAGGAAATGGA TACATTTCTGGAAGCACACAACCTC CCAAGATTGAATCAGGAAGAAATT GAAACCCTGAACAGAACAATATCAA GTTCCAAACTTGAATCAGTAATAAA AAACCTACC	Repressed in Individual 10, but not in Individual 9	AL133458
238		No insert - just vector				
239	A + AP5	No significant match to anything	93bp	AACAAGGAGATCATTTTACCATTTG CATTGTGTCAGAATAATAGACCAGA CTTCCCATTCTACAAGACACTTGAT TGAGTGCCTACTAAGCTT	Gene is repressed in Individual 10, and not expressed in Individual 9	
240	A + AP10	No significant match to anything	241bp	ATAAAGGGCCAGATAGTAGCTGTG GGCTGGGGTCTCAAACTGTGTTGC CCACTACTCAACTCTGCCATTGTAA TGTGAAAGTAGTCACAGACAAAATA TAAAGAAATGAGTGTGACTGTGTTC CAATAAAACTTTATTTACAAAAGCA TTCAGTGGGCTGGATTTGGCTTTT GGGCCATAATTAAATCCCCTCTGG NAAAATAATCACTATTTTAGCTGGA TCATGAGTACGTGGAAGCTT	Gene is repressed in Individual 10, and not expressed in Individual 9	

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
241	A + AP10	cytochrome c oxidase subunit III gene	183bp	AAGACCCTCATCAATTGATGGAGA CATACAGAAATAGTCAAACCACATC TACAAAATGCCAGTATCAGGCGGC GGCTTCGAAGCCAAAGTGATGTTT GGATGTAAAGTGAAATATTAGTTGG CGGATGAAGCAGATAGTGAGGAAA GTTGAGCCAATAATGACCGTGAAG TACGTGGAAGCTT	Induced in Individual 10, but not in Individual 9	AF004341
242	A + AP19	The size of this gene does not match the autorad	91bp	AGTAAAGGGGACAGAAGGACCTCT GAGGAGGGAAGATAGGGAGTTTGA AGCCTGAGCATTAAAGTTTCTCTGG AGTGGGAGCGATAAGCTT	Repressed in Individual 10, but not in Individual 9	
243	A + AP19	ribosomal protein S7 (RPS7)	346bp	ACTGTGAATATATACTTTTTATTTAG TCATTTTTGTTTACAATTGAAACTCT GGGAATTCAAAATTAACATCCTTGC CCGTGAGCTTCTTATAGACACCAG AAAAAGTTTCAACCTTGTGTTCCAC ATTGTTCTGCTGTGCTTTGTCCAAA TGAACCTTTATGAGCCGGCTGCCA TCTAGTTTGACGCGGATTCTCTTGC CCACAATTTCGCTTGGGAAGACCA AGTCCTCAAGGATGGCATCGTGCA CAGCTGTCAGAGTACGGCTCCTGG GACGCTTTTGCTTATTTTTTGTACG GCTTTTTCGAGTTGGCTTAAGCAGA ATTCTCCTCTGAGCGATAAAGCTT	Gene is repressed in Individual 10, and not expressed in Individual 9	NM_001011

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
243	A + AP19	No significant match to anything	392bp	GCTTATCGCTCCTAGGCTACAAAC CTGGACAGCATGTTACCGTACTGA AGACTGTAGGCAGTTGTAACACAA TGGTAAGTATATGTGTATCTAAATA TATCTAAACATAGAAGAGGTAATGC ATTGCACTATAACATTACCTTATGA CAGCCACGACATCTCTAGGTGACA GGAATTTTTTAGCTCTATTATAAGTT TCTGGGATCACCATTCTATATGTGA TCCATCATTGACTGAAATGTCATTA TGCAGCAAATTTGTATATAACTATT TCCATTGCGTCTGCCTAAAAATCTT AGTAGTGTGGGCAGAACTTGATTA GGGATTGTTTAAGGCATATATGGTC AAGCATATGAATTTCAGATATTTAT GTTTATGGAAGACTCCTTTGT	Gene is repressed in Individual 10, and not expressed in Individual 9	
244	C + AP17	No significant match to anything	125bp	AAGCTTACCAGGTAGGAGAAAATA CTAGAAAAGCACAGCTCCCTGGAG TAGTGGAATGAATCTATCATCAATA CCATTCCTACAGTTATTCTGCAATT AACATGGTACAGTAGGCAACAATTA GG	Gene is repressed in Individual 10, and not expressed in Individual 9	
245	C + AP17	clone CTA-732E4 on chromosome 22q12.1	86bp	CAACATTTGTAAAACAGGAATAAAA CCTTCCTTATATTTTTATCTCCTCTC TATCTTGAACATCTGGCATAGTACC TGGTAAGCTT	Repressed in Individual 10, but not in Individual 9	AL008722
246		Insert is too small				
247	C + AP20	UBA3 (UBA3) mRNA	133bp	CACAATATATGATTTTATTAATAAAT AGTGCAAAAGCATCAGTGATAACT GTTTGAACATTAAATTTTTTAAACAG CCATGTCTTGGCATTAGTTAATATT GTGCATATTGGCCTCTATGGCACA ACAAGCTT	Induced in Individual 10, but not in Individual 9	AF046024

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
248	C + AP21	No significant match to anything	92bp	CCAAAAAGAGCCATGCCCAGAGGG AAAGTTGGAAACGAAAGCCAAGTT TTCATTTAAAAGGAAACATTAAAGA GGTTAGCCAGAGAAAGCTT	Gene is repressed in Individual 10, and not expressed in Individual 9	
248	C + AP21	hypothetical protein (HSPC004)	96bp	CACGGAAACCAGATACATTTATTAA ATCTACTCTTAGCCGAGCAATAAAG ATGTCTACAGAGTTCACAACCTGCA ACACTTCACCAGAGAAAGCTT	Gene is repressed in Individual 10, and not expressed in Individual 9	NM_015918
249	C + AP23	Homo sapiens 12 BAC RP11-575G13	207bp	GCTTGGCTATGAGGACACAAAGGC ATAAGAATGATGCAATGGACTTTCA GGACTTGGAGGGAAGAGTGGGAG GAGGTGAAGGATAGAAGAGTACAA ATATGGTGCAGTGTATACTGCTTG GGTGATGGGTGCACCAAAATCTCA TAAATCACCACTAAAGAACTTACTT ATGTAACCAAATACCACCTGTACCT CAATAACTTATGG	Gene is repressed in Individual 10, and not expressed in Individual 9	AC010200
250	G + AP41	Familial Cylindromatosis cyld gene (hypothetical protein (HSPC057))	241bp	AAGCTTACGGGGTGTACCAAGGGA AACAGCTTTTTCAGTGTGATGAAGA TTGTGGCGTGTTTGTTGCATTGGA CAAGCTAGAACTCATAGAAGATGAT GACACTGCATTGGAAAGTGATTAC GCAGGTCCTGGGGACACAATGCAG GTCGAACTTCCTCCTTTGGAAATAA ACTCCAGAGTTTCTTTGAAGGTTGG AGAAACAATAGAATCTGGAACAGTT ATATTCTGTGATGTTTTGCC	Induced in Individual 4 and Individual 10, but not in Individual 13	AJ250014

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
250	G + AP41	ribosomal protein L8 (RPL8)	218bp	AAGCTTACGGGGTGTGGCCATGAA TCCTGTGGAGCATCCTTTTGGAGG TGGCAACCACCAGCACATCGGCAA GCCCTCCACCATCCGCAGAGATGC CCCTGCTGGCCGCAAAGTGGGTCT CATTGCTGCCCGCCGGACTGGACG TCTCCGGGGAACCAAGACTGTGCA GGAGAAAGAGAACTAGTGCTGAGG GCCTCAATANAGTTTGTGTTTATGC C	Induced in Individual 4 and Individual 10, but not in Individual 13	NM_000973
251	G + AP55	Homo sapiens chromosome 5 clone CTD-2165P17	136bp	AAGCTTACGTTAGGGGCAGAAAAG AGAAGACATTGGTTCCTATTTCACA GTGGATTGACAAAGGTGTTTCCGT AGGTAATAATGTTTTGGCTGAGAGA TGTCAGTTAGCACAGGAAGCAACA CAGTGGGATTGGCC	Induced in Individual 4, but not in Individual 13	AC010411
252	A + AP47	No significant match to anything	240bp	AGCTAAATAACCATCATTATGTTAT TAAATAGAAGCCAATTCTACCTATG TCACTCTAATTTCTTGATCTTGCAA TTTCAACTGTTGGTATTACAATATA ATTGTATTATTCATAGACAGCAAGA ATAAATAAATAAAGTGGGTGAATGA TTACATTTAAAGACATTTGTTCCTAA GTAGTAATTAGGAAAAGGCTATTAA TTAAATAATTCCTCCAAGTCACCCC ACGGGCATAAGCTT	Induced in Individual 4, but not in Individual 13	
253	A + AP47	Chromosome 16 BAC clone CIT987SK-A-67A1	106bp	AGATATAGAAGTCCCACTAGGTTG CCCTGGCTGGTCTTAAACTCTTGG TTTCAAGTGATCCTCCTGCCTTGG CCTTCCAAAATGCTGGTATTACGG GCATAAGCTT	Induced in Individual 4, but not in Individual 13	AC004531

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
254	A + AP52	hypothetical protein FLJ20436 (FLJ20436)	204bp	AAGCTTGACCTTTGTGGATGATGTT CGTTGTTCCAATCAGTCTCTTCCAA TGACCAGACACTGCCTTACCCAGA AAGATGCGACTGCCATTGGTGACC TGTTAACTCTTTACTGTGGTCTAAT GGAGACAGCACTCGACTGAAAGTG AAGGACCCAGACTCTAGTTGAACA TAAAAGGATTCCTCTGTACTAGCAC ACTCCCCT	Induced in Individual 4 and Individual 10, and only slightly in Individual 13	NM_017822
255	A + AP45	No significant match to anything	187bp	CCACAGAGTAATAATAAACTCATTC AACATTATAAAAATACTTTTCCAACT ACTTTTACATGACTTAATGTTTCCA ATTATTTGCGAAACCATGTCCCCGC AGGTCTTAGCTGGTCCTTGTTTAAG ATTCTTAATTTCATTTTGGCAATATC TAAATTTTTATTTACATACATGTGGA CTAAAGCTT	Induced in Individual 4 and Individual 10, but not in Individual 13	
256	C + AP49	clone TCBA00781	223bp	AGTTTAATAGACATTTATTCCTTCA GTTGAACAACCTCTACACAATTAAA ATGTATGACTTAAGATCTTTTCTTTT TTTGTGAAGAAATTTAGGTCTCAAG AACTTTTATGAACTTGCTATGAGTA CTTCCTGGAAATCAATTAACTGAGT CTTTTGAAACCCCTAGAGAAGATAG GAGAAAATTGGTTCAGAACGAGCA TTTAAATTAAGTCAGCCAAGCTT	Induced in Individual 4 and Individual 10, but not in Individual 13	AF283772

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
256	C + AP49	No significant match to anything	225bp	AGACAAGAGCATTGCCAGATGTAC TGTTGGATTCCCTCTTACCATGGCA AATTTCCATAGTGATTTTTATTCAGA ACGATAAACACAATTCGTTTGTGTT GATGAAAGGAAAGGGCTTGCTTGA AGTGTTTTTTGGAGTTTATAGTTTT CAACCTGCAGGAACATGTTGGATT GAGACATATAGGAAGTGATTTTTTT TTCTGTGCTTTTTAGTCAGCCAAGC TT	Induced in Individual 4 and Individual 10, but not in Individual 13	
256	C + AP49	prothymosin, alpha	224bp	AAGCTTGGCTGACAATGAGGTAGA CGAAGAAGAGGAAGAAGGTGGGG AGGAAGAGGAGGAGGAAGAAGAA GGTGATGGTGAGGAAGAGGATGGA GATGAAGATGAGGAAGCTGAGTCA GCTACGGGCAAGCGGGCAGCTGA AGATGATGAGGATGACGATGTCGA TACCAAGAAGCAGAAGACCGACGA GGATGACTAGACAGCAAAAAAGGA AAAGTTAAACT	Induced in Individual 4 and Individual 10, but not in Individual 13	M14483
257	C + AP51	KIAA0874 protein	240bp	AAGCTTCGAAATGCTAGAAAAAAAT TTGGAATGGAGTATATGCCTGAAAA GGTTTTGGATTCAGAAAGAAAAAG GATGGTTAGTTTAATCAGTGATTCT TTTTAAACTCTTCAAATATCATGAAC AAGATACTAAATTGTACCTAAGGAT TTGTATTTCTTTACAATTTGTTCTAA ATATCTGTTTAATGACTAGTTGATA TTTGTGCATGTTATTTAATAAAGAG TTATATTTTTATAG	Repressed in Individual 4 and Individual 10, but not in Individual 13	AB020681

Band	Primer Combination	Identification	Size	Sequence	Expression in Individuals Tested	GenBank Accession number
258	C + AP49	(a total of 3 matches-equal scores for each) chromosome 5 clone CTD-2284O10, Homo sapiens mRNA; cDNA DKFZp434L1,Homo sapiens cDNA FLJ12597 fis, clone NT2RM4001371	86bp	CAACAGAATAAATTATATATCCAGG AGATTCTGCCATTTTACAGCCTGGA AAAAACAATGCTTCCCTGGAAACTG GACTAAAGCTT	Induced in Individual 10, but not in Individual 13	AC008925
259	C + AP53	No significant match to anything	185bp	AAGCTTCCTCTATGTTAACACAAAC AAATGCAAATGTAGCTCTGCTTCCC TCCTTCCTTATAGTAAACACTTAAA AACCATATTTTCAATACATATGATTT GAAAATGAGATTATAACCTGCATTC TTTACTTAACAATGTATATTGCAGA CATTTTGATGCCTGTGTACAGAGAT CCTCATATG	Induced in Individual 4, but not in Individual 13	
260	C + AP56	mRNA for KIAA1618 protein, partial cds	192bp	AAGCTTATGAAGGACAGGCACAGC TGTGGACCGATTTGCAGTACAGGG AGAAAGAGGTGAAGAGATACCTGT GGCAACATCTGAAAAAACACGTGG TACCATTGCCGGACGGAAAAAGCA CGGACTTTTTGCCTGTGGACTGCC CAGTGAGGAGTAAACTGAAAACAG GCCTGATTGTCCTTTTTGTAGTGG	Induced in Individual 4 and Individual 10, but not in Individual 13	AB046838

Table 11
Class Discriminator Genes

GENE	t-test p-value
5-aminolevulinate synthase 2 (ALAS2)	0.0010
Cide-B (CIDEB)	0.0006
clone RP11-468G5	0.0008
metallothionein-IG (MT1G)	0.0040
NADH oxidoreductase subunit MWFE	0.0030
Penicillin Band 109-A-2	0.0003
Penicillin Band 117-B-2	0.0050
Penicillin Band 134-A-2	0.0031
Penicillin Band 134-A-4	0.0046
Penicillin Band 149-B-3	0.0037
Penicillin Band 239-A-2	0.0016
Penicillin Band 240-A-4	0.0000
Penicillin Band 244-A-2	0.0001
Penicillin Band 69-B-3	0.0031
Penicillin Band 77-C-2	0.0041
prothymosin, Alpha	0.0049
Rat mitochondrial cyt c oxidase I, II,III, and ATPase subunit 6	0.0029
ribosomal protein S21 (RPS21)	0.0014
ribosomal protein S24 (RPS24a)	0.0028
ribosomal protein S4, X-linked (RPS4X)	0.0008
ribosomal protein S7 (RPS7)	0.0018

CLAIMS

1. A method of identifying hypersensitivity in a subject, the method comprising:

obtaining a gene expression profile of genes associated with hypersensitivity of a subject suspected to be hypersensitive; and

detecting in the gene expression profile of the subject a predetermined pattern of gene expression of genes associated with hypersensitivity.

2. The method of claim 1 wherein the pattern of gene expression associated with hypersensitivity is obtained by comparing the gene expression profile of a hypersensitive individual with the gene expression profile of an individual who is not hypersensitive.

3. The method of claim 1, wherein the genes associated with hypersensitivity comprise at least 2 genes associated with hypersensitivity.

4. The method of claim 1, wherein the genes comprise at least 5 genes associated with hypersensitivity.

5. The method of claim 1, wherein the genes comprise at least 10 genes associated with hypersensitivity.

6. The method of claim 1, wherein the gene expression profile of the subject that is obtained comprises a profile of levels of mRNA or cDNA.

7. The method of claim 1, wherein the gene expression profile comprises a profile of levels of protein expression.

8. The method of claim 1, wherein expression of the genes predetermined to be associated with hypersensitivity is directly related to prevention or repair of toxic damage at a protein, nucleotide, macromolecule, organelle, tissue, organ or system level.

9. The method of claim 1, wherein the gene expression profile is a profile of nucleic acid expression obtained from a cell or tissue sample, or a protein expression profile derived from cells, tissues, blood, urine or serum.

10. The method of claim 1, wherein the gene expression profile is obtained from a blood, urine or serum sample.

11. The method of claim 1, wherein the method comprises identifying hypersensitivity in the subject to an agent.

12. The method of claim 11, wherein the agent is a pharmaceutical agent.

13. The method of claim 11, wherein the agent is selected from the group consisting of pharmaceutical agents listed in Table 1.

14. The method of claim 1, wherein the genes comprise genes associated with tissues or cells within the digestive system, comprising the liver, pancreas, intestines, colon, rectum, stomach, gallbladder, kidneys or bladder.

15. The method of claim 14, wherein the genes are genes associated with liver toxicity including altered lipid metabolism, fatty liver, cholestasis, jaundice, hepatitis, steatosis, necrosis, hyperplasia, mutagenesis, tumor formation or peroxisome proliferation.

16. The method of claim 1, wherein the genes are genes associated with tumor formation, teratogenesis, immunosuppression, pancreatitis, or agranulocytosis.

17. The method of claim 1, wherein the genes are genes associated with cellular manifestations of toxicity.

18. The method of claim 17, wherein the plurality of genes comprises genes associated with apoptosis, cell adhesion, autophagocytosis, cell cycle arrest, circadian rhythm, cytokine release, de-differentiation, differentiation, mitochondrial damage, migration, mutation, oncosis, peroxisome proliferation, recombination, senescence, signal refractivity, spreading, or transformation.

19. The method of claim 1, wherein the plurality of genes are genes associated with renal toxicity.

20. The method of claim 19, wherein the genes comprise genes associated with, necrosis, glomerulitis, nephritis, tumor formation, hyperplasia, proteinuria, renal damage or renal failure.

21. The method of claim 1, wherein the genes are genes associated with cardiotoxicity, blood toxicity, skin toxicity, eye toxicity or neurotoxicity.

22. The method of claim 21, wherein the plurality of genes comprises genes associated with tachycardia, arrhythmia, hypotension, hypertension, leukemia, neutropenia, agranulocytosis, peripheral neuropathy, dementia, inflammation, irritation, sensitization, myelosuppression or retinopathy.

23. The method of claim 1, wherein the genes associated with hypersensitivity are associated with a specific ethnic group, sex or age group.

24. The method of claim 1, wherein the genes are selected from the group consisting of the genes listed in Tables 3, 4, 5, 6, 8, 10 and 11.

25. The method of claim 24, wherein the genes comprises at least 5 genes.

26. The method of claim 1, wherein the genes are selected from any one of the group consisting of the genes listed in Table 4.

27. The method of claim 26, wherein the genes comprise at least 5 genes.

28. The method of claim 1, wherein the genes are expressed in one or more different cell types within a single tissue or organ.

29. The method of claim 28, wherein the tissue or organ is selected from the group consisting of liver, kidney, lung, heart, pancreas, muscle, brain, testes, ovaries, spleen, stomach, intestines, colon, rectum, eye, and bone.

30. The method of claim 29 wherein the cell types are selected from the group of liver cells consisting of Kupfer cells, sinusoidal cells, ito cells, hepatocytes, bile duct epithelial cells, hepatic venule endothelial cells and sinusoidal epithelial cells.

31. The method of claim 28 wherein the cell types are selected from the group consisting of the cells listed in Table 9.

32. A method of identifying a number of genes associated with hypersensitivity to an agent, the method comprising:

comparing the gene expression profile of cells treated with the agent with the gene expression profile of cells not treated with the agent; and

determining the genes that have altered expression due to exposure to the agent in the treated cells, thereby to identify the genes associated with hypersensitivity to the agent.

33. The method of claim 32, wherein the cells comprise cells of one or more different cell types, and wherein each said cell type comprises a gene associated with hypersensitivity to the agent.

34. The method of claim 33, wherein said cell types are derived from a single type of tissue or organ.

35. The method of claim 34, wherein said cell types are derived from an organ or a tissue selected from the group consisting of kidney, liver, lung, heart, brain, spleen, thyroid, bone, muscle, intestine, stomach or skin.

36. A method of identifying genes having a pattern of differential gene expression indicative of hypersensitivity to an agent, the method comprising:

comparing a gene expression profile of one or more cell types of a subject known to be hypersensitive to the agent with the gene expression profile of said cell types in an individual known not to be hypersensitive to the agent; and

identifying the genes from said one or more cell types having a pattern of differential gene expression, wherein the pattern of differential gene expression is associated with hypersensitivity to the agent.

37. A method of identifying genes having a pattern of differential gene expression indicative of hypersensitivity to an agent, the method comprising:

comparing the gene expression profile of one or more cell types of a subject known to be hypersensitive to the agent before treatment with the agent with the gene expression profile of the one or more cell types of the subject after treatment with the agent; and

identifying genes from said cell types having a pattern of differential gene expression, wherein the pattern of differential gene expression is associated with hypersensitivity to the agent wherein the samples of multiple individuals are compared in at least six individuals and wherein the results of gene expression profiles are compared statistically using computer software.

38. An array for the identification of a gene expression profile indicative of a hypersensitivity to an agent, the array comprising at least 25 different gene probes, each probe comprising a nucleic acid sequence of a gene associated with the hypersensitivity to the agent, wherein said gene is selected from the group consisting of genes listed in Tables 3, 4, 5 and 6.

39. An array of claim 38 wherein the array comprises at least 100 different gene probes.

40. An apparatus for identifying hypersensitivity in a subject comprising:

a detector for obtaining a gene expression profile of a number of genes associated with hypersensitivity of the subject suspected to be hypersensitive; and

a second dectector for identifying in the gene expression profile of the subject a pattern of gene expression of the genes associated with hypersensitivity, thereby to identify hypersensitivity in the subject.

41. The method of claim 11, wherein the method comprises identifying hypersensitivity in a subject to multiple agents administered together.

42. A method of determining hypersensitivity of a subject to an agent, the method comprising:

obtaining a cell from a subject;

culturing said cell to obtain a cell culture;

exposing said cell culture to an agent;

obtaining a gene or protein expression profile of a cell or cells of said exposed culture; and

detecting in said gene or protein expression profile a predetermined pattern of expression associated with hypersensitivity to the agent.

43. The method of claim 42, wherein the subject is a human being.

44. The method of claim 42, wherein the expression profile of at least 20 genes or proteins is obtained.

45. The method of claim 44, wherein the expression profile of at least 50 genes or proteins is obtained.

46. The method of claim 42, wherein the cell obtained from the subject is a leukocyte.

5

FIGURE 1

GRAPH OF GENE EXPRESSION CHANGES ASSOCIATED WITH TOXICITY OF GENOTOXIN STREPTOZOTOCIN

Gene expression profile from HepG2 cells treated with 480 µg/ml streptozotocin

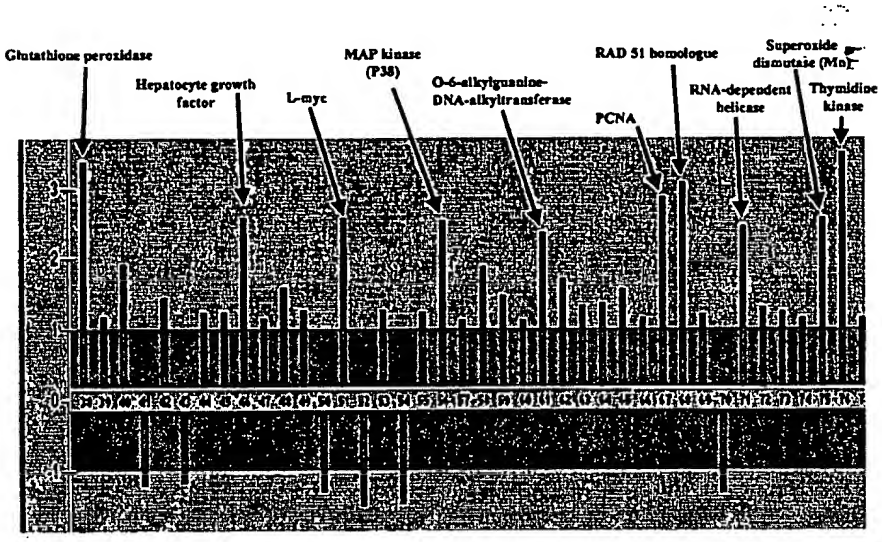


FIGURE 2

GRAPH ILLUSTRATING THE COINDUCTION OF GLUTATHIONE TRANSFERASE AND HEPATOCYTE GROWTH FACTOR RECEPTOR GENES

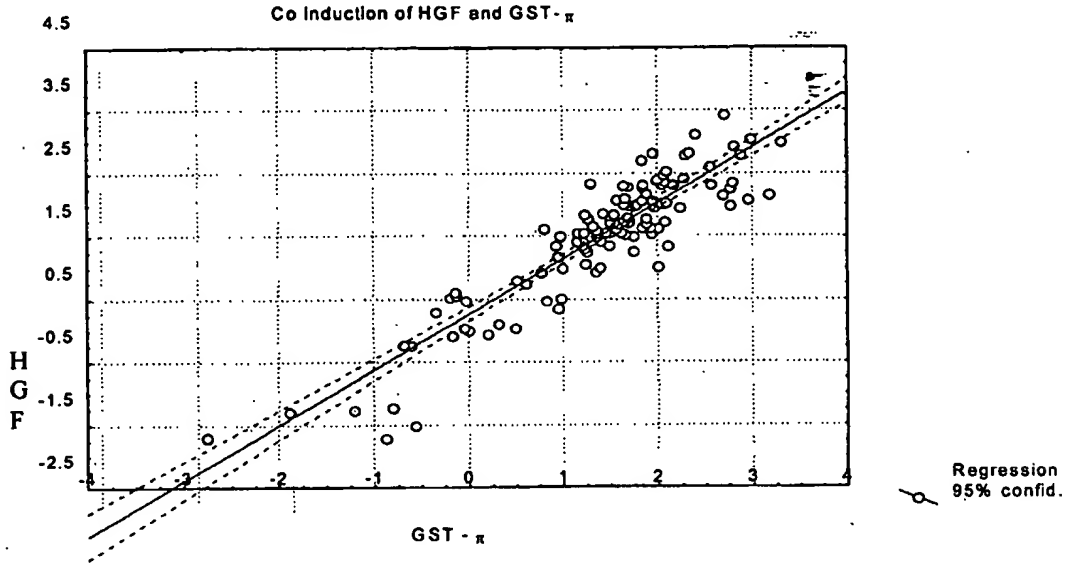
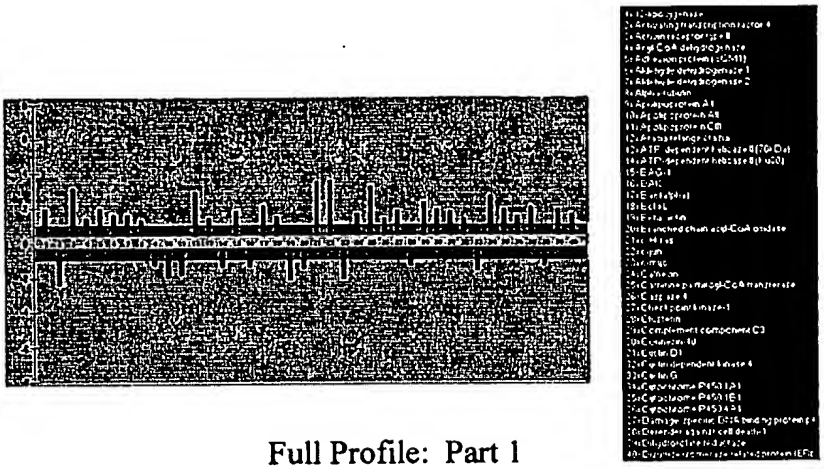
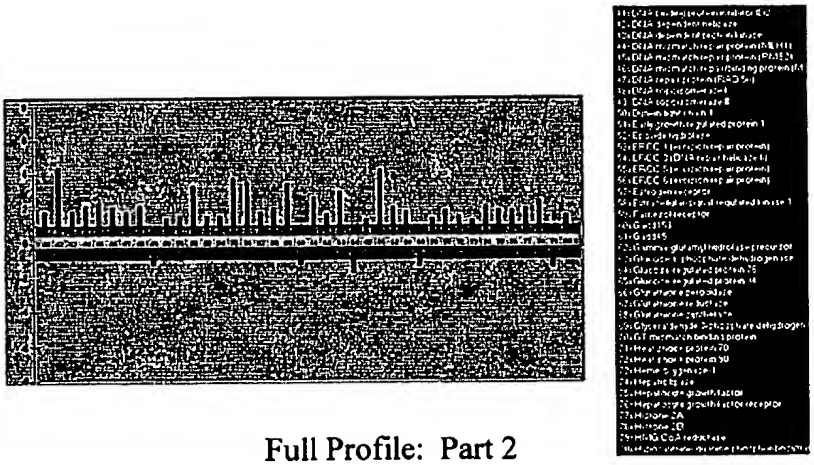


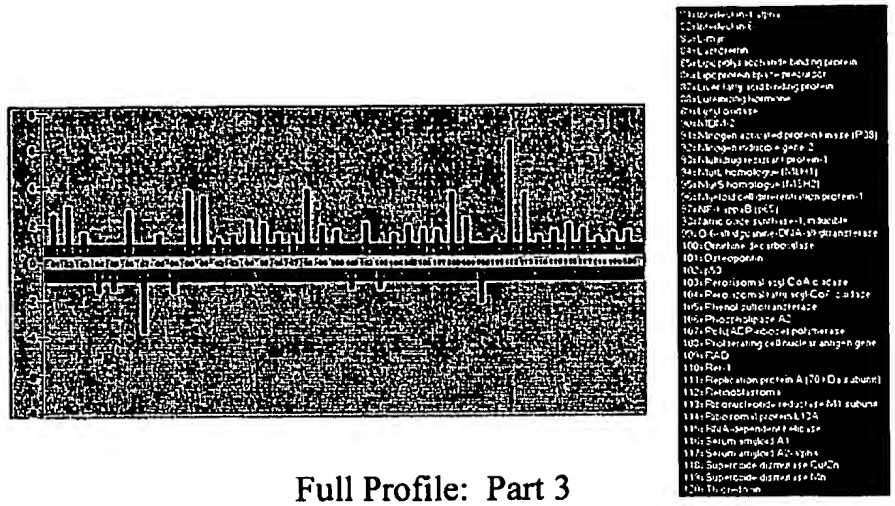
FIGURE 3
GRAPH ILLUSTRATING THE GENE EXPRESSION PROFILE FROM HEART MUSCLE AFTER EXPOSURE TO THE CARDIOTOXIN, DOXORUBICIN



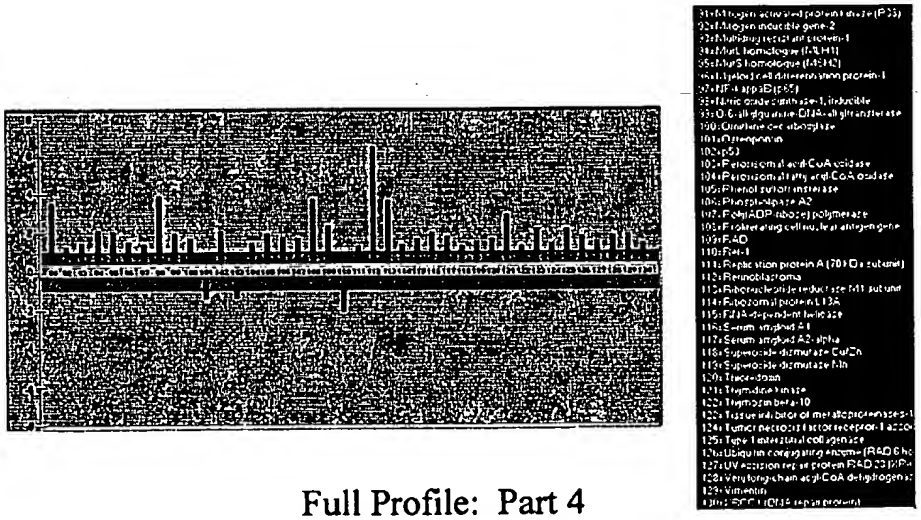
Full Profile: Part 1



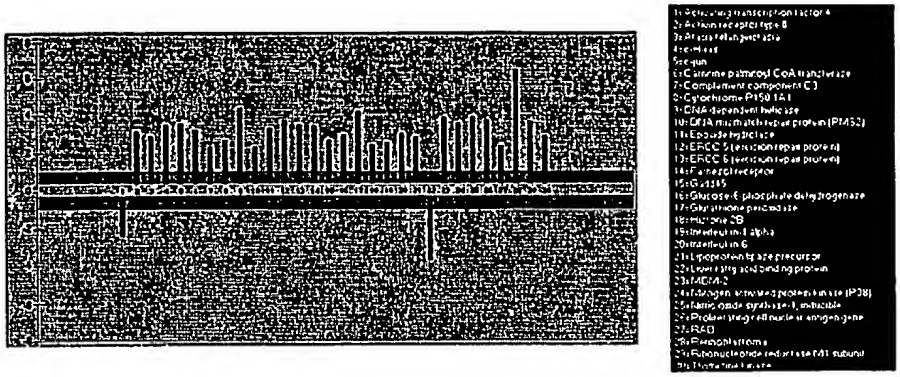
Full Profile: Part 2



Full Profile: Part 3



Full Profile: Part 4



Highest Expressing Genes Only

FIGURE 4
GRAPH ILLUSTRATING THE GENE EXPRESSION PROFILE FROM LIVER TISSUE AFTER EXPOSURE TO THE HEPATOTOXIN, WY 14643

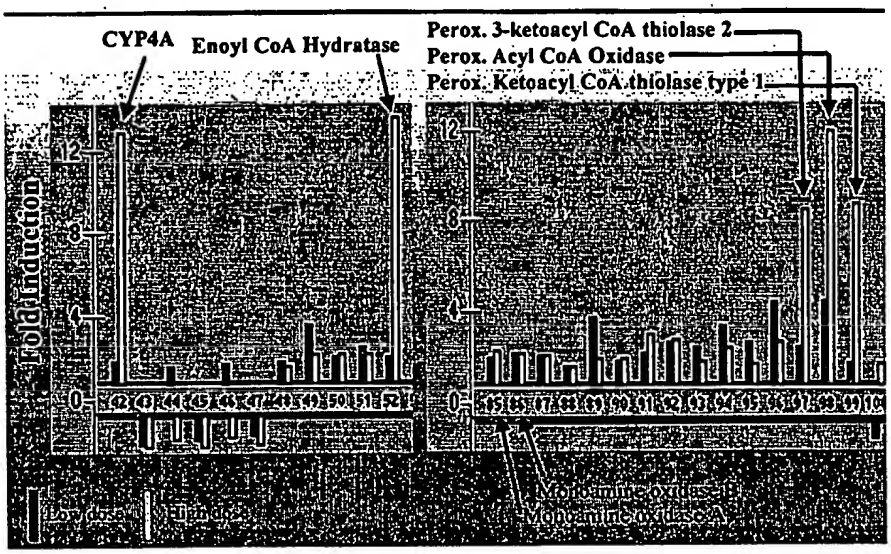


FIGURE 5
GRAPH ILLUSTRATING SOME GENES EXPRESSED FROM LIVER TISSUE AFTER EXPOSURE TO THE HEPATOCARCINOGEN CARBAMAZAPINE

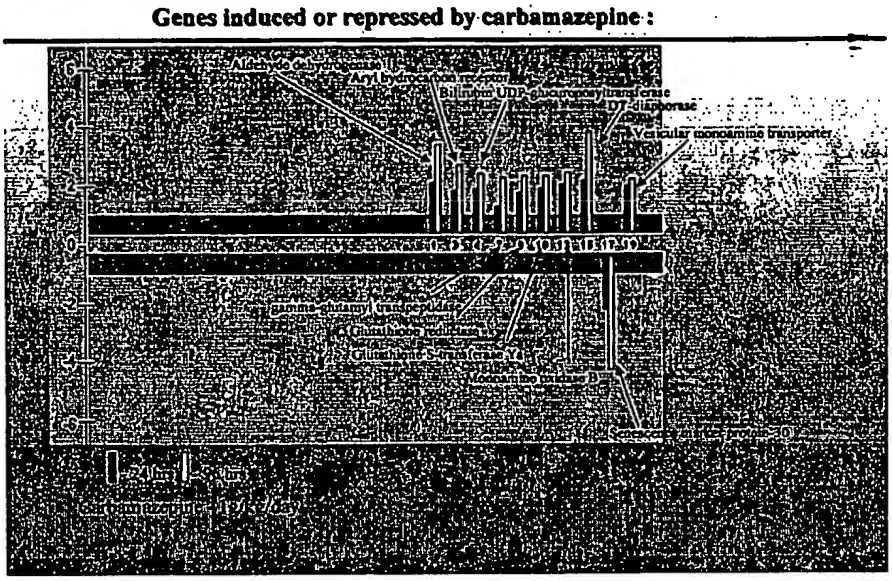


Figure 6
All gene correlation

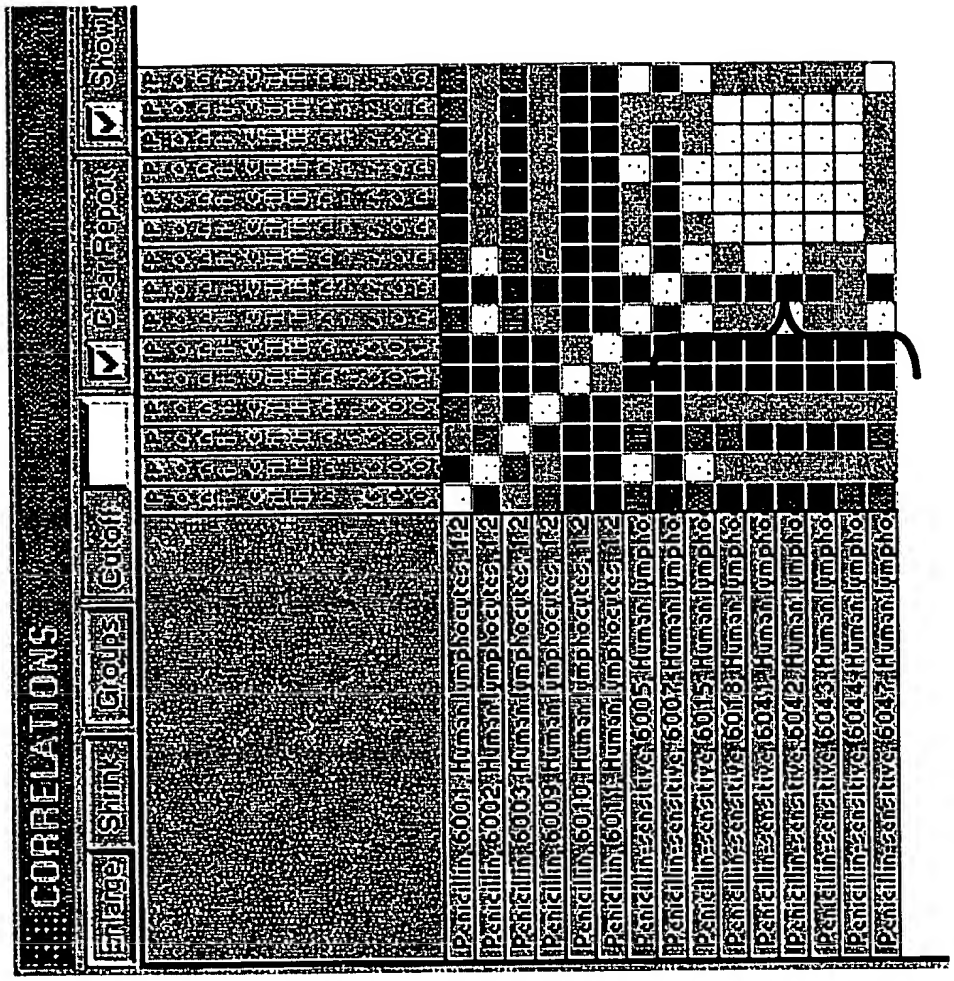


Figure 7
Discriminator Correlations

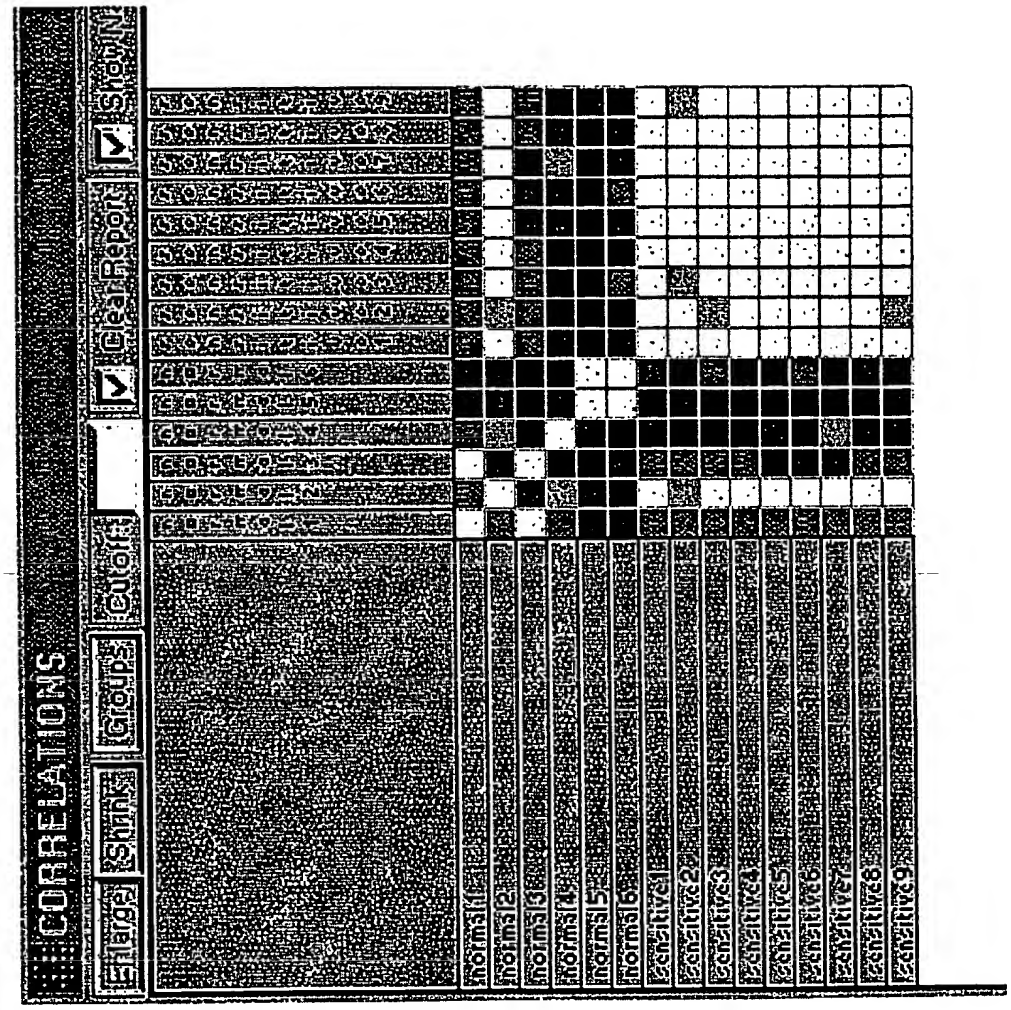
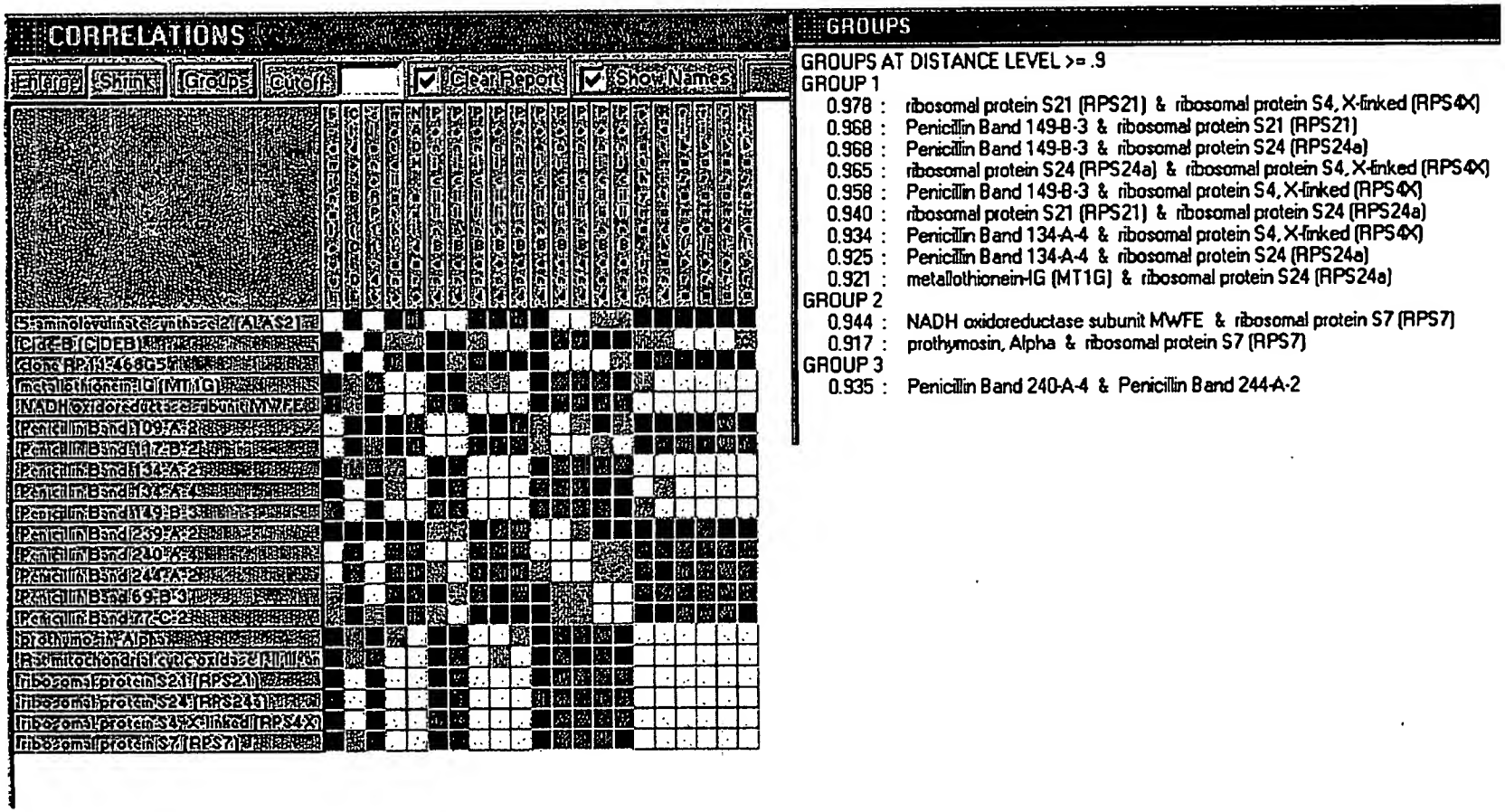


Figure 8
Inter-Gene Correlations



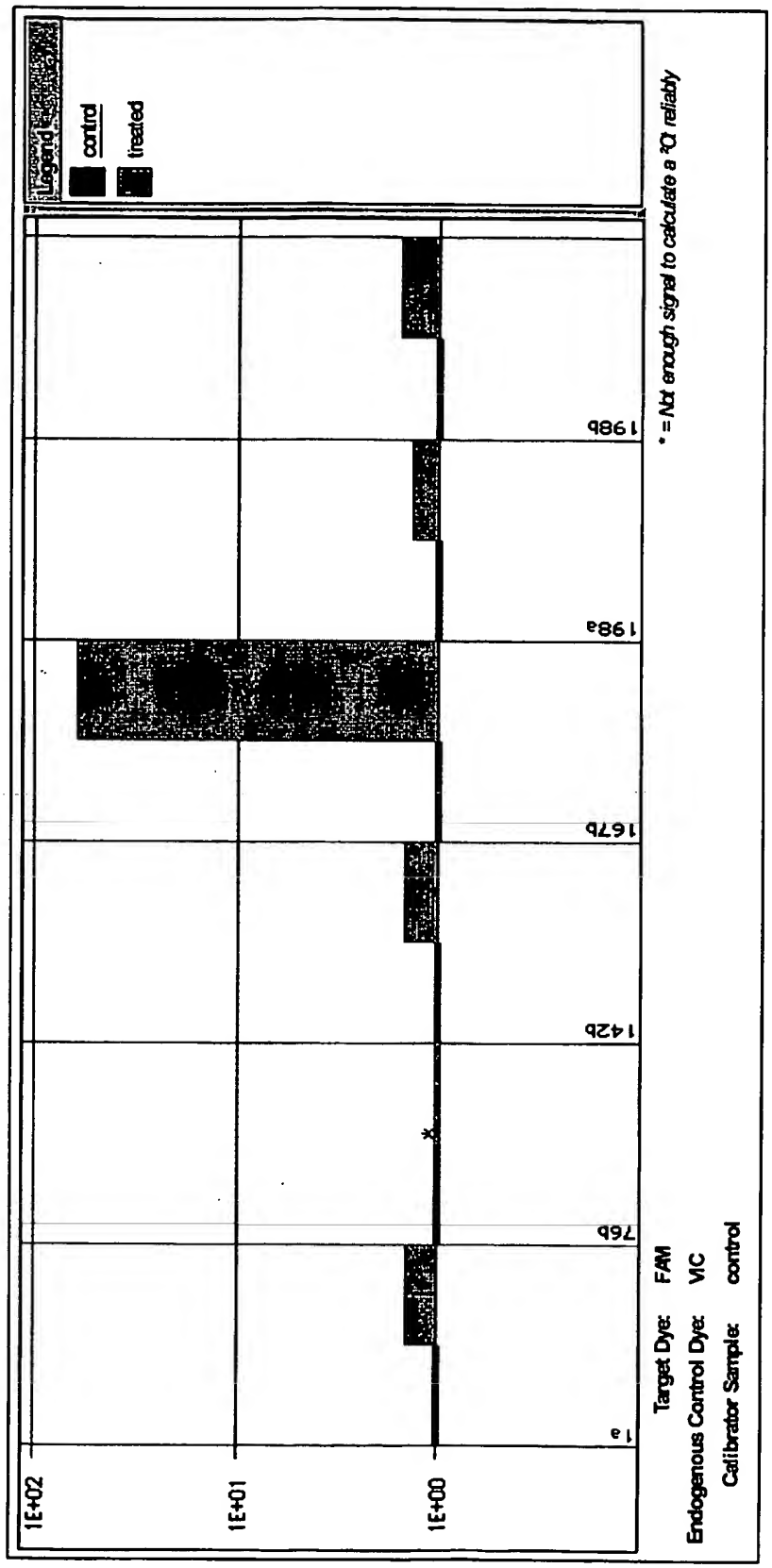


Figure 9 Taqman Results with a Penicillin Sensitive Person

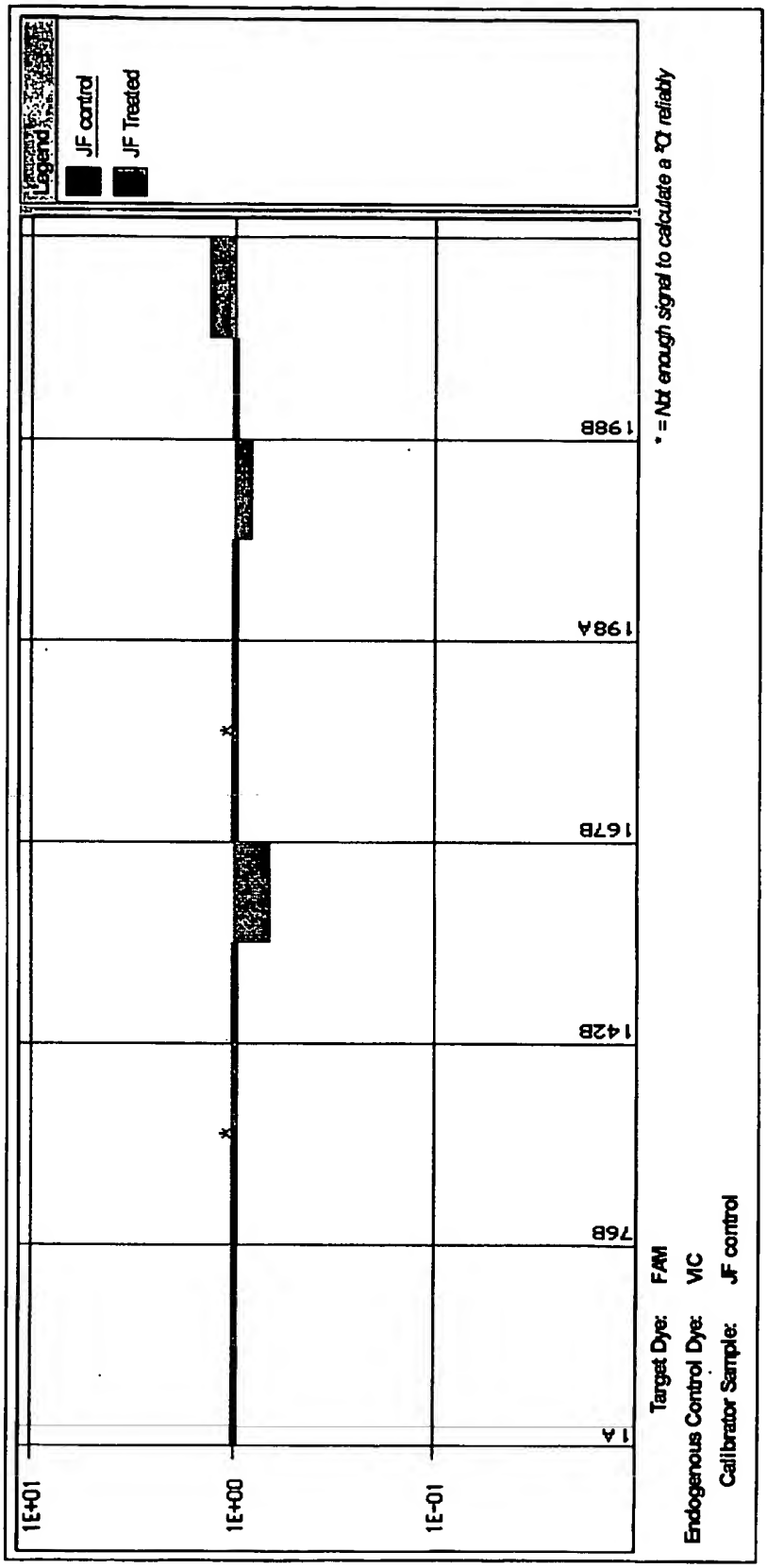


Figure 10 Taqman Results with a Penicillin Refractive Person